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REVIEW OF THE STATUS OF CHLORDANE AND HEPTACHLOR WITH
REGARD TO THEIR USE IN THE PROVINCE OF ONTARIO

PESTICIDES ADVISORY COMMITTEE
ONTARIO MINISTRY OF THE ENVIRONMENT
MARCH 1973

Date Due

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HAZARDOUS CONTAMINANTS
AND STANDARDS BRANCH
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TORONTO, ONTARIO M4V 1P5

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I SUMMARY

At the present time heptachlor is classed as a Category A pesticide, that is, it is available on a permit only basis. Chlordane is classed as either Category B or C depending on formulation and thus is readily available to agriculturalists or homeowners. Technical heptachlor contains, in addition to heptachlor, approximately 22% γ - chlordane and 5% nonachlor, both relatively persistent materials in soil. Technical chlordane contains, in addition to α -, γ - chlordane, and nonachlor, 8-10% heptachlor. On the basis of a comparison of the composition of the 2 materials, it would appear illogical to severely restrict the use of one material, while at the same time allowing widespread use of the other.

The major agricultural role of heptachlor or chlordane is for corn rootworm control. Heptachlor is effective at 1/2 lb ai/acre, chlordane usually is used at 2 lbs ai/acre. From an environmental standpoint, heptachlor epoxide residue resulting from application of heptachlor or chlordane is our chief concern. Assuming 10% conversion of heptachlor to its epoxide, the heptachlor treatment

would result in a residue of 0.037 ppm heptachlor epoxide (if calculated on the basis of a broadcast application incorporated into the top 3 inches of soil). Chlordane at 2 lb ai/acre would result in a heptachlor epoxide residue of 0.02 ppm. Thus there is some advantage in using chlordane for corn rootworm control rather than heptachlor since this treatment will generate only 1/2 the amount of heptachlor epoxide. In neither case is the residue considered significant. At the same time the use of chlordane rather than heptachlor will introduce approximately twice as much persistent γ - chlordane into the soil as well as α - chlordane. These components are persistent and are absorbed by some crops, but there is no evidence of biomagnification in milk and animal products. Data on other possible environmental effects are inadequate. On balance it would appear that there is no real environmental advantage to one material over the other insofar as rootworm control is concerned.

The second major use of heptachlor and chlordane in Ontario is for white grub control by homeowners. Many of the points noted above apply in this situation as well. However, in the case of white grub control in lawns, the insecticide is usually applied in a fertilizer mix, followed by heavy watering. It is possible that small

amounts of the insecticide would be washed from lawns and enter storm sewers. Considering the evidence presented that heptachlor hydrolizes rapidly in water to hydroxy-chlordene, which is innocuous, it would appear that in this instance heptachlor could have a slight advantage over chlordane.

The evidence submitted to the committee supports a conclusion that the current use pattern of chlordane in Ontario has not been associated with any harmful environmental effects or has produced significant residues in food or feeds.

A comparison of the metabolism and behaviour of heptachlor and chlordane support a conclusion that heptachlor could be used in a similar manner without adverse effects.

Section 1

II RECOMMENDATIONS

1. That chlordane be retained for use with its present use pattern.
2. That heptachlor be reclassified as an A category compound with exemptions to permit its use for control of northern corn rootworm on corn and soil insects on nursery and forestry crops.
3. That a research study be initiated to determine the significance of hydroxychlordene formation from heptachlor in the soil and turf in relation to Ontario climatic conditions. If hydroxychlordene is determined to be the major pathway of degradation of heptachlor, consideration should be given to utilizing this material in place of chlordane for white grub control by homeowners.
4. That a research study be initiated to identify other pesticides that will provide effective control of white grubs with no potential for deleterious environmental effects.
5. That the Provincial Pesticide Residue Testing Laboratory reinstate the milk survey in southwestern Ontario in 1973 and include chlordane residues in their assessments.

6. That laboratories monitoring pesticides in streams and lakes in Ontario include examination for chlordane residues in their assessments.
7. That monitoring of birds of prey for pesticide burden in the Great Lakes area be continued and that the methods of analysis be such as to accurately discriminate among chlordane, heptachlor and related compounds. Such monitoring should be on an annual basis to permit early detection of any significant changes.
8. That for category A and B compounds an effective procedure be developed by the Ontario Ministry of The Environment for the determination of pesticide use on a county by county basis and that this information be published.

Section 2

III REVIEW OF HEPTACHLOR, CHLORDANE AND AG-CHLORDANE

In 1969 the Provincial Government banned the use of 3 common cyclodiene insecticides: aldrin, dieldrin, and heptachlor for all uses other than termite control by licensed exterminators. The use of technical chlordane, a related cyclodiene insecticide was not restricted. In 1971 the Pesticides Advisory Committee was requested to review the chlordane-heptachlor problem. Two questions were posed to the committee: 1) In the light of new information on the persistence of heptachlor in soil and water, was its banning justified; and 2) In the light of new information on the composition, behaviour, and persistence of technical chlordane in soil is continued use of this compound still justified? The Pesticides Advisory Committee agreed that the situation warranted review and a subcommittee was assigned this responsibility. The subcommittee arranged a symposium of invited experts in order to obtain the most up-to-date information. This symposium was held on November 15, 1972. Submissions of the speakers are appended to this report (Appendices 1-13).

A) Federal Registrations and Use of Heptachlor and Chlordane in Ontario.

1) Heptachlor

At the Federal level, several uses of heptachlor were considered acceptable for registration as of January 1, 1971, i.e. for control of wireworms on wheat, oats, barley, and rye; corn rootworms and cutworms attacking corn; wireworms and cutworms attacking tobacco; white grubs, European chafer, chinch bugs, earwigs, and ants attacking lawns, turf, and golf courses; and control of the narcissus bulb fly. At the Provincial level, heptachlor was banned in 1969. Information presented by the Velcisol Corporation on heptachlor use is given in Appendix 7.

2) Chlordane

Acceptable claims for registration of technical chlordane at the Federal level as of January 1, 1971, were more extensive than those for heptachlor. Chlordane is registered on a wide variety of crops, lawns, turf, and nurseries for control of white grubs, wireworms cutworms, European chafer, Japanese beetle, and white-fringed beetle. It is registered for corn rootworm control, for root weevils on strawberries and ornamentals, for sod webworm on lawns, for earthworms on

golf courses, and for a variety of insect problems relating to flowers and bulbs. It is also registered for a wide variety of insect pests involving spot treatment in dwellings and non-food industrial plants; for spot treatment on outdoor surfaces of buildings for control of insects; and for soil treatment near building foundations for termite control.

At the Provincial level there are several uses for chlordane, primarily for control of soil pests. Its major use is for control of the corn rootworm. Many entomologists in Ontario have mixed feelings concerning efforts to control this pest. Surveys have indicated that quite a large amount of the corn acreage does not need to be treated. The corn rootworm is a pest which can be controlled by cultural methods, i.e. crop rotation. The tendency in Ontario, partly for economic reasons, has been to encourage "continuous corn". In addition, the widespread use of persistent herbicides makes it impossible to rotate corn with herbicide-susceptible crops the following year. As a result, the corn rootworm is becoming established in the southwestern counties of the province. Prior to their being banned, aldrin and,

to a lesser extent, heptachlor were the materials of choice for rootworm control. Since 1969, one material which has received extensive use is chlordane. Information from Statistics Canada for agricultural insecticides for the years 1968-70 reflect (on a Canada-wide basis) the increased use of chlordane in the absence of aldrin (Table 1). More specific information provided to this committee on a confidential basis by the Velsicol Corporation is given in Appendix 7.

Table 1. Sales of aldrin and chlordane
1968-70 (Canada wide).

Year	Aldrin (lbs)	Chlordane (lbs)
1968	183,057*	22,613*
1969	127,034*	39,058*
1970	40,919	100,524*

* Incomplete data due to confidentiality requirements of the Statistics Act.

Chlordane is preferred by many growers because of its low cost and low toxicity. However, there is a possibility that the corn rootworm is becoming resistant to chlordane, as has occurred in the United States, and if this is the case, its use will decrease in the next few years. Several alternative organophosphorus and carbamate insecticides are registered and recommended for corn rootworm control in Ontario. However, they are more expensive and nearly all more toxic than chlordane.

A second agricultural use of chlordane is for wireworm control. Wireworms are primarily pests following sod, but they can be pests following sod for up to 2 or 3 years. Chlordane is the most

effective material available at present for wireworm control. Two other alternative insecticides are available. The use of chlordane for wireworm control in Ontario at present is very limited. Wireworm populations are low, and presumably will remain depressed until dieldrin and/or DDT residues in soil drop below bioactive levels. At that time populations will begin to increase. There is some evidence that this is beginning to occur in Ontario.

A third use of chlordane in Ontario, which involves both the homeowner and to a lesser extent agriculture and forestry, is for control of white grubs, Japanese beetle, and European chafer. At present the only recommendation for control of these pests is chlordane. The Velsicol Corporation has provided an estimate of the amount of chlordane sold in Ontario in 1972 primarily for homeowner use for white grub control (Appendix 7). There is no safe, effective alternative insecticide which can be placed in the hands of the homeowner for white grub control in lawns. It was estimated that about 1500 acres of potatoes are treated each year. It was also noted that sod farms are treated with chlordane where there is a grub problem. In addition, sod (or other

nursery stock) to be exported to the United States must have been treated with chlordane within 2 years of export. Another problem with nursery stock involves control of the black vine root weevil.

Again chlordane is the only insecticide available for its control, although its efficacy is questionable. In addition to white grubs, the Japanese beetle has appeared as local infestations in isolated areas in Ontario. This extremely destructive pest of lawns, golf courses, etc., is controlled jointly by Federal and Provincial agencies any time an infestation is discovered. Chlordane is used at 6 lb/acre. Over the last four years acreage treated has not been extensive. Use of chlordane in forestry in Ontario is limited to white grub control in nurseries and outplantings. Currently about 500 acres per year are being treated in outplantings in eastern Ontario for white grub control. Thus chlordane has a small, but crucial role in forestry in Ontario.

Chlordane has had and still has extensive use in Ontario for structural extermination. Prior to the build-up of resistance it was used to control practically all structural pests, but it is now used

mainly to control termites, and to a lesser extent ants, sowbugs, millipedes and centipedes. The Ontario Pest Control Association feels that there is no adequate substitute available for termite control.

3) AG-Chlordane

AG-Chlordane (code no. HCS-3260) is a "cleaned up" chlordane comprising 98% alpha + gamma chlordane. At present it is undergoing extensive evaluation as an experimental soil insecticide. There are no registered uses for this material in Canada.

B) Composition of Heptachlor, Chlordane, and AG-Chlordane

1) Heptachlor

Heptachlor was originally isolated from technical chlordane. Chemically it is 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene. Technical heptachlor, i.e. the material available to the grower, comprises about 73% heptachlor, 21-22% γ -chlordane, and approximately 5% nonachlor.

2) Chlordane

This material was first manufactured in 1946 and the pure chemical compounds now called "chlordane" in the chemical sense, were the first substances isolated

from it. So it became known as chlordane. It was originally thought that the pure product was about 90% chlordane. There was some disagreement as to the actual chlordane content and it was subsequently agreed that "technical chlordane" should be considered to have 60-75% chlordane content. By the mid-60's it became apparent that even this conclusion was in error. The two compounds known as chlordane, i.e. α -chlordane and γ -chlordane, comprise a minority of the very complex mixture in technical chlordane. Heptachlor is also a component of technical chlordane. The four known insecticidal components of technical chlordane are heptachlor, α -chlordane, γ -chlordane, and nonachlor. Recent analyses on technical chlordane conducted by the Agriculture Canada Research Institute, London, indicate the following (Table 2).

Table 2. Insecticidal components in technical chlordane.

Insecticide	%	
	Reference grade	Emulsifiable concentrate
γ -chlordane	10.5	11.6
α -chlordane	8.6	10.4
heptachlor	8.5	9.4
nonachlor	ND ¹⁾	2.7
	27.6	34.1

1) Not determined.

3) AG-Chlordane

Ag-chlordane has been prepared in small amounts for field evaluation. It consists of about 95% α -chlordane and γ -chlordane in the ratio of 3:1 alpha:gamma. This experimental product also has a specification that it should not contain more than 1% heptachlor or nonachlor. Recent analysis of reference grade AG-Chlordane by the Agriculture Canada Research Institute, London, indicate the following composition: α -chlordane, 68%; γ -chlordane, 29.7%; heptachlor, 1%; and nonachlor, 0.6%.

C) Biological Activity and Fate of Heptachlor, Chlordane, and AG-Chlordane in Soil

1) Heptachlor

Heptachlor is probably the most effective soil insecticide ever available for agricultural use. Like all soil insecticides its behaviour is moderated by soil and climatic factors. Generally, it was recommended for control of a wide range of soil insects at rates of 0.5 to 1.5 lbs ai/acre, although some early recommendations were as high as 5.0 lbs ai/acre. It was introduced in Canada in the early 1950's and by 1959 the first indications of soil insect resistance began to appear. Between 1959 and 1967 a number of species of root maggots and cutworms developed a high level of resistance. At present the only major species of soil insects which are not resistant in Ontario to heptachlor are the corn rootworm, white grubs and wireworms.

In soil, heptachlor itself is only moderately persistent. It volatilizes from soil and this is probably the major route of disappearance. However, a small amount of heptachlor is converted by microbial action to heptachlor epoxide. This compound is more persistent and is absorbed from soil by some crops.

In addition, technical heptachlor, as noted above contains about 22% γ -chlordane. The latter can be classed as a highly residual insecticide.

Heptachlor was banned in Ontario in 1969 on the basis of evidence that heptachlor epoxide was formed in soil and it was thought at that time that the epoxide was as persistent in soil as dieldrin. More recent evidence has indicated that the pathways of degradation of heptachlor in soil are much more complex. It has been determined that in water and to a lesser extent in soil the major pathway of degradation of heptachlor is to an innocuous hydrophilic material, 1-hydroxychlordene, and that 1-hydroxychlordene will in turn degrade to 1-hydroxy, 2,3 epoxychlordene. A minor pathway of degradation is to another innocuous compound, chlordene, which in turn can be degraded to chlordene epoxide. Some heptachlor epoxide is also formed in soil but it has been shown that heptachlor epoxide is also metabolized to 1-hydroxychlordene. These results, first demonstrated in the laboratory at the Agriculture Canada Research Institute, London, Ontario, have been confirmed by field studies in the United States and the Maritimes. However, the field studies in the United States have indicated that the extent of

heptachlor conversion in soil to hydroxychlorde-
ne is dependent on rainfall. The greater the rain, the
greater the conversion to hydroxychlorde-
ne. No field data has been accumulated in Ontario to determine the
significance of the hydroxychlorde-
ne degradation under Ontario conditions. In water, con-
version of heptachlor to hydroxychlorde-
ne is rapid and it would appear unlikely that heptachlor itself could
be a serious water pollutant. The possibility does
exist, however, that the small amounts of heptachlor
epoxide or γ -chlorde-
ne in soil resulting from the
initial application of heptachlor would move by surface
erosion into streams and rivers. Under these conditions,
it is likely that heptachlor epoxide would be slowly
converted to hydroxychlorde-
ne. However, some of the
available epoxide would probably be picked up by aquatic
organisms and passed up through the various trophic
levels. The fate of γ -chlorde-
ne is not clear. There is
limited evidence that it will biomagnify in some
aquatic organisms (molluscs) but not to as great an
extent as heptachlor epoxide.

2) Chlordane

In contrast to heptachlor, technical chlordane is only a moderately effective insecticide. Laboratory bioassay studies indicate that it is about 1/8 as toxic in soil as heptachlor. Consequently it has been used at higher rates of application of from 1-10 lbs ai/acre. As with heptachlor its behaviour in soil is moderated by soil and climatic factors. Recent studies have indicated that the majority of the insecticidal activity (60-80%) of technical chlordane is due to its approximately 8% heptachlor content.

As would be expected, the persistence of biological activity of chlordane in soil parallels that of heptachlor, i.e. it is only moderately persistent. However, some of the components of chlordane are highly residual in soil although below the bioactive level. Small amounts of the heptachlor component will be metabolized to the more persistent heptachlor epoxide. Laboratory studies have indicated that nonachlor, α -chlordane, and γ -chlordane may be classed as highly persistent. The terminal residues of chlordane in soil are α -chlordane and γ -chlordane. A comparison of the relative amounts of persistent residues in the

soil as a result of a broadcast application of heptachlor or chlordane for white grub control might be useful at this point in the discussion (Table 3).

Table 3. Estimated residues of persistent insecticides in soil as a result of heptachlor or chlordane treatments for white grub control.

Insecticide	lb ai/ acre ¹⁾	ppm in soil				
		hepta- chlor	hepta- chlor epoxide ²⁾	α -chlor- dane	γ -chlor- dane	nona- chlor
heptachlor	1.5	1.17	0.12	0.00	0.33	0.08
chlordane	8.0	0.75	0.08	0.83	0.93	0.22
AG-Chlordane	8.0	0.08	< 0.01	5.44	2.38	0.05

1) Amounts required to obtain an equivalent degree of control.

2) Estimating 10% conversion of heptachlor to heptachlor epoxide.

3) AG-Chlordane

Laboratory studies have indicated that, in terms of initial biological activity, AG-Chlordane is slightly less effective than technical chlordane. However, because of the preponderance of α -chlordane and γ -chlordane in the product, it is much more persistent. The use of AG-Chlordane would reduce heptachlor and heptachlor epoxide residues in soil to a minimum, but high levels of persistent

α - and γ -isomers would be added to the soil (Table 3). Preliminary field studies in British Columbia and Ontario appear to confirm the laboratory findings that α -chlordane and γ -chlordane are persistent in soil.

D) Metabolism of Heptachlor, Chlordane, and AG-Chlordane in Plants and Animals

1) Heptachlor

As noted above, heptachlor epoxide is a minor, but significant degradation product of heptachlor in soil. Heptachlor epoxide is absorbed and translocated by some crops. The amount of absorption is dependent on the concentration of the residue in soil, soil and climatic factors. The major factor is soil type, specifically organic content of the soil. The higher the organic content, the less the absorption of the residue. In Ontario the highest residues of cyclodiene insecticides such as aldrin/dieldrin and heptachlor/heptachlor epoxide are found in muck soils. In these soils the residues are so strongly tied up that little in the way of residues is found in crops. Problems which have arisen (mainly aldrin/dieldrin) have occurred in mineral soils. In the case of heptachlor/heptachlor epoxide, studies by the Provincial Pesticide Residue Testing

Laboratory and the Health Protection Branch of the Department of Health and Welfare have never indicated any serious problem in Ontario with residues of heptachlor/heptachlor epoxide in crops used for human consumption.

Heptachlor epoxide is also absorbed under the conditions noted above by crops used for animal feed. Here the problem is potentially more serious since heptachlor epoxide, if ingested by animals, will likely occur at higher levels in the fat of those animals if uninterrupted feeding takes place, than the level of intake. In other words, it is an amplifier. Such amplification (an average of 5 times the level ingested) can lead to unacceptable levels of heptachlor epoxide in milk and animal products. Information obtained by the Health Protection Branch indicates that between October 1, 1969 and March 31, 1970 44 samples of dairy products were analyzed and 27 contained heptachlor epoxide (61%) at levels of 0.01-0.1 ppm. After the ban on the use of heptachlor of 56 samples taken between July 1, 1971 and December 1971, 12 contained heptachlor epoxide (21%) at levels of 0.01-0.06 ppm. Thus levels in dairy products appear to have dropped slightly. During the same period 31 samples of meat

products were analyzed of which 29 were negative. There was also little evidence of heptachlor epoxide in feed samples. Data obtained by the Provincial Pesticide Residue Testing Laboratory between 1967-69 indicated that heptachlor epoxide was found in milk samples as follows: southern Ontario (along Lake Erie), 3.5%; western Ontario (along Lake Huron), 10.7%; central, eastern, and northern Ontario, 0%. Residues found in milk could be correlated with the use of heptachlor as a seed grain treatment and for maggot control in turnips. In 1970-71, no residues of heptachlor epoxide were found in the southern region although almost 100% of the samples contained dieldrin. Very low levels of heptachlor epoxide were found in meat and meat products in 1969-70 and have decreased since then.

2) Chlordane

Terminal residues of technical chlordane, i.e. heptachlor epoxide, α -chlordane and γ -chlordane, are also absorbed by some crops at rates dependent on concentration, soil and climatic conditions. Heptachlor epoxide derived from soil applications of chlordane does not appear to be a problem. Numerous studies have indicated that heptachlor epoxide rarely exceeds 1% of the terminal residues. Chlordane residues

do not tend to biomagnify in mammals and the "Storage Concentration Ratio" does not exceed 1 and is generally <1. Lipophilic metabolites found in animal fat are rapidly converted to hydrophilic metabolites which are excreted in the urine. Total diet studies in the United States and Canada have indicated that chlordane is not a significant dietary contaminant.

3) A - chlordanes

Terminal residues of AG-Chlordane would be α -chlordane and γ -chlordane. It is likely that higher residues of these compounds would be found in agricultural crops if AG-Chlordane was introduced for use. No significant biomagnification in mammals would be likely.

E) Environmental Effects of Heptachlor and Chlordane

1) Heptachlor

In the soil ecosystem heptachlor/heptachlor epoxide have shown no significant effects on non-target soil microorganisms. However, their effects on beneficial non-target soil animals are more severe. They are moderately toxic to earthworms. In addition earthworms absorb significant amounts of these materials, which can result in

deleterious effects on birds. As would be expected, heptachlor/heptachlor epoxide are toxic to a wide spectrum of soil arthropods and their use results in drastic reductions in populations of such beneficial insects as predatory beetles and collembola. Other insecticides, including many of the new organophosphorus and carbamate insecticides have similar deleterious effects on soil animal populations. However, to keep the situation in its proper perspective, it should be noted that research in Great Britain has established that normal cultivation has more drastic effects on non-target soil animals than normal applications of either aldrin/dieldrin or heptachlor/heptachlor epoxide. Studies conducted at the Provincial Pesticide Residue Testing Laboratory and the Agriculture Canada Research Institute, London, have indicated that agricultural soils in Ontario generally contain little or no heptachlor or heptachlor epoxide. However, in locations with a history of heptachlor use, residues of γ -chlordane are often detected.

There is considerable evidence which indicates that heptachlor is highly toxic to birds. In areas of the United States where heptachlor was used on a

wide scale for eradication of the fire ant, bird populations were reduced. It has also been demonstrated that seed-eating birds are affected by heptachlor-treated seed. In Ontario use of heptachlor in eradication programs was never adopted. Seed treatments were used for many years, but no serious effects were noted. Studies by the Provincial Pesticide Residue Testing Laboratory on samples of brain from birds indicated no significant heptachlor epoxide residues with the exception of ring-necked pheasants collected in Lambton County which were less than 0.1 ppm.

Studies conducted by the Provincial Pesticide Residue Testing Laboratory and the Agriculture Canada Research Institute, London, have indicated no significant levels of heptachlor or its epoxide in stream water or sediments. Studies conducted by the Canada Centre for Inland Waters, Burlington, in 1970 and 1971 indicated no significant accumulation of heptachlor or its epoxide in water or sediments in Lakes Ontario, Erie or Huron. No significant levels of heptachlor epoxide were found in fish taken from streams or the Great Lakes.

Considering this data, surprising results were obtained in a study conducted by the Canadian Wildlife Service which indicated significant residues of heptachlor

epoxide in newly-hatched chicks of several species of fish-eating birds collected from Lakes Ontario, Erie, and Huron. Residue levels were reported to be as high as 0.75 ppm. The information provided also indicated that residues of heptachlor epoxide increased in adult birds throughout the summer. In view of the fact that environmental levels of heptachlor epoxide are so low in Ontario, the data obtained by the Canadian Wildlife Service is unexpected. However, with the exception of one species of bird (herring gull), the others are all migratory and pass the winter in the southern parts of the United States and around the Gulf of Mexico, where pesticide residue levels are known to be much higher. The migratory habits of the birds may explain, in part, this discrepancy. In addition, the possibility of analytical error must be considered. In addition to residues of "organochlorine insecticides", the birds also contained very high levels of PCB's (e.g. heptachlor epoxide 0.3 ppm; PCB 316 ppm). It is well-known that analysis of heptachlor epoxide and some other organochlorine insecticides is very difficult in the presence of PCB's. Preliminary studies at the Agriculture Canada Research Institute, London, on collaborative samples provided by the Canadian Wildlife Service indicate that the amounts of heptachlor epoxide in the chicks may have been seriously overestimated.

2) Chlordane

In the soil ecosystem the effects of chlordane are similar to those obtained with heptachlor. Chlordane has no significant effect on soil microorganisms but will affect non-target soil animals. It is toxic to earthworms at high rates of application and is, in fact, registered for control of earthworms on golf courses. When used at higher rates of application its effects on non-target soil arthropods parallel those obtained with heptachlor. Data (pre-1970) obtained by the Agriculture Canada Research Institute, London, and the Provincial Pesticide Residue Testing Laboratory has not indicated any significant build-up of chlordane residues in agricultural soils in Ontario. However, it should be noted that the use of chlordane was limited (Table 1) until 1970 when it became a major replacement for aldrin and heptachlor.

To date, none of the laboratories monitoring pesticide residues have detected significant levels of chlordane in water or sediments in streams, rivers, or the Great Lakes. Information on the environmental impact of chlordane is limited. Generally speaking, it would appear that it does not exhibit a propensity to magnify in the environment.

Section 3

 CANADA DEPARTMENT OF AGRICULTURE PRODUCTION AND MARKETING BRANCH PLANT PRODUCTS DIVISION	DATE Ottawa, Ont. October 9, 1970	NUMBER T-66
	Pesticide	

MEMORANDUM

Re: Heptachlor Uses Acceptable for Registration Under the Pest Control Products Act

The uses of heptachlor that are acceptable for registration in Canada have been reevaluated in the light of current information and requirements. The attached summary lists those uses that are acceptable for registration as of January 1st, 1971.

Labelling of currently registered products containing heptachlor must be revised to agree with the accepted uses, cautions and limitations set out in the attached summary. Revised draft labelling for products containing heptachlor must be submitted before registration can be granted for 1971.

NOTE: The Minister of Agriculture may permit additional uses for specific situations where essential need has been demonstrated.

JAS/pm

HEPTACHLOR

Common Name: heptachlor

Chemical Name: 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene

Formulations: Du dust
EC emulsifiable concentrate
Gr granular
Sn solution

Guarantee in terms of: heptachlor

Classification: insecticide

PRECAUTIONARY LABELLING

Hazardous Properties: Heptachlor is poisonous if swallowed, inhaled or absorbed through the skin.

Symptoms of Poisoning: Nausea, vomiting, hyperirritability and convulsions.

Precautions: Poison and "skull and crossbones" on concentrated formulations. Keep out of reach of children. Avoid skin contact, inhaling or swallowing. Keep in original container during storage. Destroy empty container. Handle and apply only as recommended and at recommended rates. Do not apply or allow to drift to areas occupied by unprotected humans, or beneficial animals, or food crops. Do not contaminate feed or foodstuffs. Do not store near feed or foodstuffs. Do not contaminate streams, lakes, ponds, irrigation water, water used by livestock or water used for domestic purposes. Wash thoroughly with soap and water after handling and before eating or smoking. If clothing contaminated, wash immediately and clean before re-use. During spray mixing and loading operations, wear clean synthetic gloves and a mask or respirator of a type suitable for heptachlor protection.

First Aid: Call a doctor in case of accident. If swallowed, cause vomiting by drinking salt water or by inserting finger in throat. For eyes, flush with water. If on skin, wash promptly with soap and water.

Note to Physician: Treatment. The administration of barbiturates is beneficial. Avoid adrenaline or morphine. Oxygen may be indicated.


Limitations:

1. Do not make any soil applications for crops grown in a rotation which includes root crops likely to be used for animal feed.
2. Do not graze or feed or sell for feeding to livestock any part of the treated crop, crop refuse or crop by-product.
3. Do not use higher rates than listed. If adequate control is not obtained with these rates, use an alternative insecticide.

ACCEPTABLE CLAIMS FOR HEPTACHLOR FORMULATIONS FOR REGISTRATION
UNDER THE PEST CONTROL PRODUCTS ACT

Host or Location	Pest or Use	Dosage Rate (active ingredient) and Formulations	Directions for Use
wheat, oats, barley, rye	wireworms	.5 oz. per bushel Du, Sn	<u>Seed treatment:</u> Apply to thoroughly coat each seed.
corn	corn rootworm	8 oz. per acre EC, Gr	<u>Soil treatment:</u> Apply a six inch band in the seed row at planting. If these directions are followed closely, all portions of the harvested plant may be fed to livestock. Do not graze dairy animals in the corn field after harvest. Limitations (1) (3)
	cutworms	24 oz. per acre EC, Gr	<u>Soil treatment:</u> For use as broadcast application to soil before planting. Disc into soil immediately. If these directions are followed closely, all portions of the harvested plant may be fed to livestock. Do not graze dairy animals in the corn field after harvest. Limitations (1) (3)
tobacco	wireworms	2 oz. in 40 gallons of water EC	<u>Soil treatment:</u> Apply mixture as transplant water. Use 160 gallons of mixture per acre. Limitations (1) (3)
	cutworms	24 oz. per acre EC, Gr	<u>Soil treatment:</u> Apply spray to surface of soil before planting crop. Limitations (1) (3)
lawns, turf, golf courses	white grubs, European chafer, chinch bugs, earwigs, ants	60 oz. per acre EC	<u>Soil treatment:</u> Apply in spring or when insects are first observed. For grubs, water into soil. Limitations (1) (2) (3)

Host or Location	Pest or Use	Dosage Rate (active ingredient) and Formulations	Directions for Use
narcissus	narcissus bulb fly	32 oz. in 100 gallons of water EC	Soak bulbs in mixture for ten minutes before planting
		60 oz. per acre EC	<u>Soil treatment:</u> Use 18 inch band application over the row at planting before covering with soil. Limitations (1) (3)

 <p>CANADA DEPARTMENT OF AGRICULTURE PRODUCTION AND MARKETING BRANCH PLANT PRODUCTS DIVISION</p>	DATE Ottawa, Ontario. September 9, 1970	NUMBER T-63
	pesticide	

TRADE MEMORANDUM

Re: Chlordane Uses Acceptable for Registration Under the Pest Control Products Act

The uses of chlordane that are acceptable for registration in Canada have been reevaluated in the light of current information and requirements. The attached summary lists those uses that are acceptable for registration as of January 1st, 1971.

Labelling of currently registered products containing chlordane must be revised to agree with the accepted uses, cautions and limitations set out in the attached summary. Revised draft labelling for products containing chlordane must be submitted before registration can be granted for 1971.

Note: At the discretion of the Minister of Agriculture and upon the advice of the Federal Interdepartmental Committee on Pesticides, uses other than those included in the summary may be allowed for public health or plant quarantine purposes where no suitable alternative is available.

JAS/dm

Replaces Memorandum T-30, dated May 27, 1968.

CHLORDANE

Common Name: chlordane

Chemical Name: 1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane

Formulations: Du dust
EC emulsifiable concentrate
Gr granular
PS pressurized spray
Sn solution
WP wettable powder

Other Names: octachlor, chlordane

Guarantee in terms of: technical chlordane

Classification: insecticide

PRECAUTIONARY LABELLING

Hazardous Properties: Harmful if swallowed. Skin contact may cause toxic symptoms. Toxic to fish and wildlife.

Precautions: Avoid breathing dust or spray mist. Avoid skin contact. Wash with soap and water after using. Avoid contamination of food, feed, drinking water and utensils. Do not contaminate lakes, streams or ponds. Do not apply to livestock. Do not use on cats. Do not apply in dairy barns or poultry houses. Keep out of reach of children.

First Aid: For skin contact, wash with soap and water. If in eyes, flush with plenty of water. If swallowed, induce vomiting. Get prompt medical attention.

Note to Physician: The administration of barbiturates is beneficial. Avoid use of adrenaline and morphine. Oxygen may be indicated.

Limitations:

1. Do not apply to green or spring onions.
2. Do not apply more than once every four years, or more than once in a crop rotation cycle.
3. For use in regulatory and control programs of the Plant Protection Division of the Canada Department of Agriculture.

ACCEPTABLE CLAIMS FOR CHLORDANE FORMULATIONS FOR
REGISTRATION UNDER THE PEST CONTROL PRODUCTS ACT

Host or Location	Pest or Use	Dosage Rate (active ingredient) and Formulations	Directions for Use
beans, corn, lettuce, onions, red beets, radishes, potatoes, sweet potatoes, broccoli, kale, brussels sprouts, cabbage, cauliflower, cucumbers, cantaloupe, tomatoes, strawberries	wireworms, white grubs, subterranean cutworms	5-10 lbs. per acre EC, Gr	<u>Soil treatment:</u> Broadcast application to soil before planting. Work into top 3-6 inches of soil. Use higher rates in heavy, muck soils only. Limitations (1) (2)
	wireworms, white grubs	1-2 lbs. per acre EC, Gr	<u>Soil treatment:</u> Band application 8 inches wide over row at planting time placed one inch above seed and in front of press wheel but not in contact with seed. Limitations (1) (2)
corn	corn rootworm	1-2 lbs. per acre EG, Gr	<u>Soil treatment:</u> Band application 7 inches wide over row at planting time.
strawberries	strawberry root weevil	5-10 lbs. per acre EC, Gr	<u>Soil treatment:</u> Apply to soil before planting. Work into top 3-6 inches of soil.
ornamentals, shrubs, flowers, bulbs	root weevils	5-10 lbs. per acre EC, Gr	<u>Soil treatment:</u> Apply to soil and work into top 3-6 inches before planting.
	lygus bugs, earwigs, ants, thrips, cutworms, caterpillars, armyworm, bulb flies	1-3 lbs. per acre Du, WP, EC	Treat soil or foliage as required to control pests. Do not apply during bloom. For bulb flies, soak bulbs in 5% water solution before planting.
lawns	white grubs, wireworms, ants	5-10 lbs. per acre Du, WP, EC, Gr	<u>Soil treatment:</u> Apply to soil of lawns. Water into soil after treatment.
	sod webworm	4-5 lbs. per acre Du, WP, EC, Gr	<u>Soil treatment:</u> Apply to established grass only. Do not water into soil.
greens on golf courses	earthworms	8 oz. per 1000 sq. ft. WP, EC	<u>Soil treatment:</u> Apply to heavily infested areas of turf on golf greens. Water into soil where applied. Do not use this rate near streams, lakes or ponds. For application by experienced pest control operators only.

Host or Location	Pest or Use	Dosage Rate (active ingredient) and Formulations	Directions for Use
nurseries, soil and turf	European chafer, Japanese beetle, White-Fringed beetle	5-10 lbs. per acre Gr	<u>Soil treatment:</u> Apply to soil of nurseries or where turf is being grown. Water into soil after treatment. Apply to transportation sites where turf is being shipped. Limitation (3)
spot treatment in dwellings and non-food industrial plants	cockroaches, bedbugs, fleas, earwigs, silverfish, ants, carpet beetles, clothes moths, crickets, spiders, box elder bugs, gnats, mosquitoes, wasps, bees, stable flies, stored product insects such as confused flour beetle, larder beetle, saw-toothed grain beetle	2-3% spray EC, Sn, PS 5% dust Du	<u>Surface treatment:</u> Use spot applications to surfaces and behind fixtures where infestations occur. For fleas spray or dust kennels and animal bedding. Do not apply to animals or plants. Remove all pets including fish from area before treating. Do not allow treatment to contaminate food, feed, or utensils. Do not treat when industrial plants are in operation. Do not treat clothing, bedding or furniture. Do not apply to surfaces that may contact food. Never use as a space spray.
spot treatment on outdoor surfaces of buildings	mosquitoes, gnats, wasps, bees, flying moths	2% spray EC, Sn, PS	<u>Surface treatment:</u> Apply to limited areas near windows, doors and light fixtures outdoors on dwellings and other buildings. Do not apply to plants or animals. Never use as a space spray.
soil treatment near foundations of buildings	subterranean termites	5-10 lbs. in 100 gallons of water EC	Apply to treatment to soil at rate of one gallon per lineal foot of trench dug next to building foundations.

A

chlordane
DWELLINGS

July/Aug/71

brown dog tick

2-3% solution Sn
5% dust Du

Apply as spot treatment to control infestations.
Repeat if necessary.

R

LAWNS

ants, European chafer,
Japanese beetle, white
grub, wireworm

5-10 lb per acre Du EC

Treat at anytime except when the ground is frozen.
Treat between late-July and mid-September for
control of European chafer. Do not apply on
newly seeded lawns. If reseeding, wait one month
after treating soil. Against ants, dust on ant
hills and repeat as necessary. After treatment,
sprinkle the area thoroughly. Do not graze
livestock on treated areas or use clippings as
feed.

chlordane

February 7, 1972

R

NON-FOOD INDUSTRIAL
PLANTS AND DWELLINGS

ants, bees, bedbugs,
booklice, box-elder bug,
brown dog tick, carpet
beetle, centipede,
clothes moth, cock-
roach, cricket, ear-
wig, fleas, grats,

2-3% solution Sn
5% dust Du

Apply as spot treatment to surfaces and behind
fixtures when infestations occur. For fleas,
spray or dust kennels and animal bedding. Do not
apply to animals or plants. Remove all pets in-
cluding fish from area before treating. Do not

millipedes, mosquito,
silverfish, sowbug,
spiders, springtails,
stable fly, stored-
product insects
(i.e., confused flour
beetle, larder beetle,
saw-toothed grain beetle),
and wasps

allow treatment to contaminate food, feed, or
utensils. Do not treat when industrial plants are
in operation. For bedbugs, treat bed frames. Do
not treat clothing, bedding or upholstered furniture.
Do not apply to surfaces that may contact food.
Never use as a space spray.

NEEDS FOR CHLORDANE-TYPE PESTICIDES
IN SOIL INSECT CONTROL

by

F. L. McEwen

November 15th, 1972

Agricultural needs for chlordane-type pesticides are largely confined to soil pests of turf, lawn and sod areas, or, for agricultural uses in crop production in areas where sod has recently been broken. In the agricultural area, the problems are mostly related to three insect pests, namely: white grubs, wireworms, and the northern corn root-worm.

White Grubs

Chlordane is the only material recommended for control of white grubs in Ontario. It is recommended at the rate of 5 lbs per acre and is used to a significant extent in potato production. Most white grubs have a life cycle that lasts about three years. The eggs are laid preferably in sod or grass areas and the larvae that hatch from these eggs will be feeding for the following two years. Thus if potatoes are planted on an area that has been in sod in either of the past two seasons, it is possible that a white grub problem may develop.

Wireworms

These are troublesome pests in a number of our agricultural crops. The adults known as click beetles are not especially destructive but the insect has a long life cycle and depending on species, this cycle can be two to six years. The adults lay the eggs in sodded areas and problems with the larvae can be experienced up to several years after breaking this sod. Chlordane has been a recommended insecticide for control of wireworms and potatoes at a dosage rate of 5lbs per acre. In this case, there are two other materials registered for use. These are: fonofos and carbofuran. In 1972 these insecticides were each recommended at dosage rates of 5 lbs per acre. It is believed that the rate with carbofuran can be reduced and used as a band treatment at the rate of 3 lbs per acre. This method of application for carbofuran will be the only one recommended in 1973.

Northern Corn Rootworm

By far the largest amount of chlordane used in agriculture is used for the control of the northern corn rootworm. This insect is a pest of debatable significance in that infestations tend to be erratic in nature and difficult to predict. The insect is a pest of corn in the southwest part of the province, but it is only a pest where corn is grown after corn. This happens because the adult insect will lay its eggs only around corn plants and these eggs are laid in mid-to late summer; young larvae hatching the following Spring will then feed on corn replanted in the same area. It has been recommended in Ontario for many years that a rotation be used so that corn does not follow corn. When this is practised, no problem arises due to the northern corn rootworm. Grower practice, however, has moved rather consistently toward a pattern of growing corn after corn. This is dictated by the general economics of corn production and the fact that the most effective and widely used herbicide in corn may cause some problems when other crops are grown in the corn rotation. This is due to persistence of the herbicide, especially during dry seasons to be phytotoxic to plants such as beans seeded there the following year.

During the past two years resistance to chlordane on the northern corn rootworm has developed in some parts of the United States, and in Ontario there are reports of unsatisfactory control. It may be that chlordane will be phased out of northern corn rootworm control programs on the basis of decreasing efficacy of resistance develops further. There are a number of alternative materials that are recommended for control of the northern corn rootworm. These include: Bux, 4-8, and chlorfenvinphos. Diazinon has also been recommended but is normally not considered as effective.

The use of chlordane in northern corn rootworm control is a confined use. The method of treatment is normally to use a granular material which is placed in the seed furrow at planting time. Thus risk of contamination through air currents, etc., is largely minimized. In addition, the insecticide is placed in an area where it is not likely to be exposed to non-target organisms other than those resident in the soil.

There are some uses of chlordane related to agriculture in a sense - the use in lawns, golf courses, etc., for control of such pests as white grubs, European chafer, and Japanese beetle. Any of these pests can be extremely damaging if numbers are large in a sodded area. The Japanese

beetle has been controlled through a systematic approach carried out jointly by the Federal and Provincial Agencies. In any area where the Japanese beetle is detected, it is now routine to make an application of chlordane at the rate of 6 lbs per acre. Over the past several years, a limited acreage has been treated almost each year. A thousand acres was the largest treatment any single year. The Japanese has appeared in a number of widely separated areas of the province during the past several years. It would appear that treatments to contain this pest have been successful, and with this history it is likely that they will be continued.

The European chafer is present in many areas of New York State and is a constant threat to southern Ontario. Should infestations develop, chlordane would be the material of choice for use.

CHLORDANE USE BY THE MINISTRY OF NATURAL RESOURCES

by

K. B. Turner

November 15th, 1972

Within the pest control program of the Ministry of Natural Resources, the use of chlordane at the present time is quite small. It is used only to control white grubs. There are two separate situations where white grubs may be a problem --- in nurseries, and in field outplantings of areas being reforested.

In the ten nurseries which are currently in production, white grubs are a potential problem on a total potential annual acreage of 200, that is, the acreage seeded each year. However, experience shows that grubs are a significant problem only in those nurseries located in certain agricultural areas, and currently we are treating with chlordane only about 50 acres annually. The recent trend toward more rototilling of beds prior to planting has also reduced the grub problem.

While white grubs present a continuing but relatively small maintenance problem in three or four nurseries, a larger problem exists in the reforestation program where the rehabilitation of abandoned farm land is involved. In most instances these farms have a heavy sod cover, and grubs can be a very significant problem. The problem was particularly severe about ten years ago, and in many areas plantations were doomed to failure in the absence of chlordane treatment at time of planting. However, in recent years the grub problem has dropped off very noticeably, with the result that only about 500 acres of reforested land is treated annually, and most of this acreage is in southeastern Ontario (Leeds and Grenville counties) where the soil is shallow over limestone. The current trend in field treatments is away from liquid formulation and toward granular material. A few granules are placed in the hole as each tree is planted. Total dosage rate is 4-5 lbs ai/acre. Treatments are not routine but are based on evidence of grub problem as revealed by the presence of significant numbers of June beetles the Spring prior to planting. Because of the geographic overlap in the three basic broods of white grubs in Ontario, and in the overlap

of development of individuals within populations, it is becoming very difficult to plan control operations on the basis of the traditional three-year life cycle.

Although grub populations are low at present, the insect is cyclical and will undoubtedly return in infestation proportions in future. Under such conditions chlordane or similar material is vital in being able to protect trees during the first, second or third crucial years after planting. One treatment at planting-time is sufficient; an important point because retreatment is exceedingly difficult and expensive.

CHLORDANE USE IN THE NURSERY BUSINESS

by

K. Laver

November 15th, 1972

One of the main uses of chlordane in this area has to do with the production of sod on sod farms. In Ontario, we have large sod farms of an average size of approximately 500 acres. Where a white grub problem exists, it is necessary to treat and growers are pretty much restricted to the use of chlordane.

In a broader context, the nurseryman is faced with a problem in order to export nursery stock or any material with earth attached to the roots. In order that we may export products with soil attached into the United States, it must be certified that an application of a pesticide has been used for the control of certain soil pests. Aldrin, dieldrin and heptachlor were previously used for this purpose, but with the banning of these materials in Ontario, the material available now is chlordane. Previously, an application rate of 10 lbs per acre of chlordane was required to export. This rate has now been reduced to 5 lbs. Chlordane is the only material that will be accepted as adequate treatment for nursery stock for export and it must be certified that the application has been made at a specific rate within two years of export.

Nurserymen are in general agreement that chlordane is not as effective as were the materials: aldrin and dieldrin. There is also concern that when the amount of dieldrin in the soil from previous applications has been reduced through degradation, new problems with soil pests in the nursery may appear. Such insects as white grubs and the black vine root weevil may present problems.

ONTARIO PEST CONTROL ASSOCIATION

A NON-PROFIT ORGANIZATION



November 28, 1972

Mr. Keith Laver
Chairman
Pesticides Advisory Committee
5th Floor, Mowatt Block
Queen's Park
Toronto, Ontario

Dear Sir:

I wish to thank the Committee for the opportunity of presenting the position of our Association on Chlordane and Heptachlor.

As I stated at the meeting, it is our position that all pesticides and herbicides should be controlled to prevent abuse or over use, but that the benefits of all chemicals should be available to the people of Ontario under regulated conditions and being applied by experts. We have never felt that banning the use of any chemical was beneficial, but before any substance is used full research into its potential benefits and dangers to the environment must be carried out and only then released for use by professional applicators, licensed by the Province.

As I stated, heptachlor has had very limited if any use in structural extermination in Ontario. Chlordane (technical) has had and still has an extensive use. Prior to the build up of resistance chlordane was used for the control of practically all structural pests but now it is used mainly for the control of termites and to a lesser degree the control of ants, sow bugs, millipedes and centipedes. It is also being used in a very limited way by some companies as a cheap method of servicing monthly accounts, particularly if no serious infestation is present. Our Association is not in accord with this latter practice and partly because of this are embarking on a complete review of our methods of service, with the aim of eliminating the unnecessary use of any pesticide.

With reference to termite correction and prevention services it is our opinion that chlordane at present has no substitute. It is the least hazardous to the operator, and its effects are the most long lasting in protecting buildings against invasion by termites. As I stated tests in Mississippi have shown that the protection by chlordane applied more than twenty years ago are still giving 100% protection. This is due to a heavy dosage in a very limited area in and around a building and the fact that chlordane does not leach out of the soil but generally stays where we put it and thus presents little if any environmental effect.

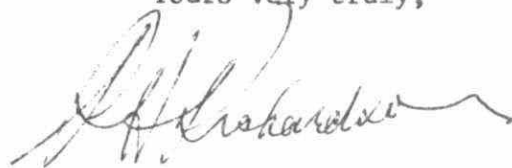
Mr. Keith Laver

In this following paragraph I will be expressing conclusions of myself and not of our Association, based on the presentations of the other speakers at the meeting and I ask your indulgence if I am out of line.

I have concluded that Heptachlor has a definite benefit in agriculture but must be strictly controlled. I would suggest that it be used under permit and that a condition of the permit that a record of the dosage used on land be kept by the applicator. To prevent dangerous levels of residues in the soil I would suggest that the dosage of the first year on any land be reduced to one-half or even one-third on any successive application. This should ensure effective use and at the same time prevent a build-up in the soil of the more hazardous components of Heptachlor.

On behalf of the Ontario Pest Control Association, I again wish to thank you for this opportunity.

Yours very truly,



P. H. Richardson
ONTARIO PEST CONTROL ASSOCIATION
Executive Secretary

PHR:ds

copied for Dr. McEwen

COMMENTS BY DR. P. B. POLEN (VELSICOL CHEMICAL CORPORATION)
TO
ONTARIO PESTICIDES ADVISORY COMMITTEE MEETING,
WED., NOV. 15, 1972, TORONTO, ONTARIO, CANADA

VELSICOL CHEMICAL CORPORATION

COMMENTS BY DR. P. B. POLEN (VELSICOL CHEMICAL CORPORATION)
TO
ONTARIO PESTICIDES ADVISORY COMMITTEE MEETING,
WED., NOV. 15, 1972, TORONTO, ONTARIO, CANADA

Composition of Chlordane, Heptachlor and AG Chlordane, and the History of Their Development

Technical chlordane is a multicomponent organochlorine insecticide which was first developed in about 1946. It derived its name at that time from two components (α - and γ -chlordanes -- $C_{10}H_6Cl_8$) which were first isolated from it. The name was chosen by joint agreement at a meeting in 1947 of representatives of two agencies of the United States Government (Department of Agriculture and Food & Drug Administration) and the two manufacturers (Velsicol Chemical Corporation and Julius Hyman & Company). (At present Velsicol Chemical Corporation is the only commercial producer of technical chlordane.) At that time the product was considered to contain 60-75% of the chlordane isomers and was so labeled. As we now know, the pure molecular species called chlordane comprise less than one-third of the total composition of the technical product. Heptachlor ($C_{10}H_5Cl_7$) is a minor constituent of technical chlordane, and has been reported in the literature to comprise between 5 and 9% of that product. Among other constituents of technical chlordane are four isomers of chlordene ($C_{10}H_6Cl_6$) and nonachlor -- also referred to as "enneachlor" ($C_{10}H_5Cl_9$). Technical chlordane consists of 64-7% organochlorine and is standardized since about 1950, to conform to the biological, chemical and physical properties of Reference Technical Chlordane.

In reviewing technical chlordane, one should consider on the basis of its own properties. It would be misleading to characterize technical chlordane as having the properties of any one of its individual components, heptachlor for example, without giving due regard to its total composition and interaction of all of its constituents. Even more

misleading would be the attribution to technical chlordane of properties of one or two other members of the chlorinated hydrocarbon family which resemble it in formal rather than characteristic ways. I shall endeavor to bring together, in a nutshell, the observations from the literature which will assist you in making a valid evaluation.

Technical chlordane is the only commercial chlordane now produced. An experimental product referred to as HCS-3260 or AG Chlordane, has been distributed in small quantities for experimental evaluation. That product consists (95%) of two isomers of chlordane in the approximate ratio of 3:1 (alpha:gamma); the heptachlor content does not exceed 1% and typically is below 0.5%. This product must at present be regarded as a new one requiring evaluation of efficacy and safety -- as is now being done in supervised field trials throughout the world. At present Velsicol supplies commercially only technical chlordane.

Another related commercial product is technical heptachlor which typically consists of about 73% heptachlor, 22% γ -chlordane (trans-isomer) and 5% nonachlor.

Metabolism of Chlordane, Heptachlor and AG Chlordane in Plants and Animals

Under the aegis of the Commission on Terminal Pesticide Residues, International Union of Pure and Applied Chemistry (IUPAC), a series of studies clarified the knowledge about terminal residues of chlordane. It was determined that α - and γ -chlordanes constitute the principal components of the terminal residues resulting from treatments of crops with technical chlordane. Even considering the presence of heptachlor as a constituent of technical chlordane, the Commission concluded that in general heptachlor and heptachlor epoxide were insignificant constituents, if present at all, in the terminal residues from treatments of technical chlordane. Portions of the IUPAC Commission's Reports dealing with chlordane are collected and presented as Exhibit VI.

The terminal residues of AG Chlordane would also be the two principal isomers of chlordane. Heptachlor and nonachlor would, of course, be absent or negligible.

Heptachlor, in the context of a minor component of technical chlordane, apparently reacts differently than is generally regarded as its behavior as the dominant constituent in technical heptachlor. The rapid conversion of heptachlor to the non-toxic 1-hydroxychlordene is illustrated in a study of the "aging" of residues of technical chlordane by Bevenue and Yeo [J. Chromatog., 42, 45-52 (1969)] -- see Exhibit I. In this study, gas chromatography was employed. It is an analytical technique which is capable of following the fate of the individual constituents. The preferential conversion of heptachlor to 1-hydroxychlordene, rather than to heptachlor epoxide, is made very clear in this study. This helps to explain the absence (or insignificance) of heptachlor and heptachlor epoxide in the terminal residues resulting from technical chlordane treatments. Not only is 1-hydroxychlordene less toxic than either heptachlor or its epoxide, but it is also less persistent and does not magnify in the food chain.

The compound heptachlor, as it occurs from applications of technical heptachlor, converts on plants primarily to 1-hydroxychlordene through hydrolysis, but a minor proportion is converted to heptachlor epoxide. Heptachlor epoxide is -- by comparison -- more stable and may biomagnify and for these reasons engenders some concern in regard to toxicological potential and environmental impact. Heptachlor epoxide is also very readily detected analytically and has gained much more attention for this reason than other metabolites.

The focus of concern, therefore, is heptachlor epoxide which may be formed in varying degrees from applications of either technical chlordane or heptachlor, depending upon the conditions. It should be kept in mind, however, that the transformation of heptachlor to epoxide is a relatively minor pathway even for heptachlor in its concentrated form, and this transformation of heptachlor is generally of lesser importance as a result of application of technical chlordane. This conclusion is reinforced by hundreds of residue analyses

which we have performed on crops at harvest after growth in chlordane-treated soils where it was found that heptachlor epoxide rarely exceeds about 1%, if present at all, in the terminal residues.

Technical chlordane does not exhibit a propensity to magnify in the environment, as a number of monitoring studies show. For example, see a review of the literature by Clive A. Edwards ["Persistent Pesticides in the Environment", Critical Reviews in Environmental Control, 1 (1), 7-67 (1970)]. Several tables have been excerpted from Edwards' article and are presented as Exhibit II. Your attention is drawn to the entries in the columns headed "Concentration factor" -- an index of magnification. It is evident from the comparison of the values listed for chlordane, which are approximately unity, with those for other organochlorine compounds, which may reach 5- and 6-figure values, that chlordane shows little, if any, tendency for magnification in the environment.

Chlordane does not tend to biomagnify in mammals. This may be seen in a study of animal metabolites of chlordane [Polen, et al., Bulletin of Environmental Contamination & Toxicology, 5 (6), 521-528 (1970)] -- Exhibit III. In this study it is observed that the "Storage Concentration Ratio" -- similar to the "Concentration factor" discussed above as a measure of magnification -- does not exceed about 1. when rats or dogs are fed chlordane virtually an entire lifetime; cattle or swine fed for shorter periods exhibits Storage Concentration Ratios of substantially less than 1. This is consistent with a report of Boyd on a field study [Bulletin of Environmental Contamination & Toxicology, 5 (4), 292-299 (1970)] -- Exhibit V. Chlordane residues on alfalfa were compared with "(apparent) heptachlor epoxide" (probably largely oxychlordane) levels in milk and it was found that an average ingestion level of 3.549 parts per million chlordane in hay resulted in 0.551 ppm (average) of (apparent) heptachlor epoxide in the fat of milk. We calculate the "Concentration factor" to be 0.155, a value substantially below 1. and indicative of non-biomagnification.

In addition to the lipophilic metabolites for chlordane cited above -- metabolites found in the fat of animals --

chlordanes are largely and rapidly converted to hydrophilic metabolites which are readily excreted in the urine. Among the conversion products isolated are a chlorohydrin and a dihydroxy derivative of chlordanes which result from replacement of one or two of the organochlorine atoms by hydroxyl groups. The ease with which these conversion products are excreted explains the non-biomagnification of chlordanes.

Schematic presentations of the pathways of transformation of chlordanes and heptachlor are presented as Exhibit VII which are copies from the 1970 Evaluations of Some Pesticide Residues in Food: The Monographs, Report of the Joint Meeting of Food and Agriculture Organization (FAO) and World Health Organization (WHO) Pesticide Residue Experts meeting in Rome in November, 1970 (AGP: 1970/M/12/1).

Total diet studies in the United States provide additional insight as to the impact, if any, of use of chlordanes (and heptachlor). Chlordanes are not a contaminant in the American diet. Its level of occurrence is too low to quantitate. This is reflected in the 2nd Annual Report of the President's Council on Environmental Quality (August, 1971), Table A-6 of which is attached as Exhibit IV. Ten compounds are listed therein. These are evaluated against the FAO/WHO Index of Safety known as Acceptable Daily Intake (ADI), that is "...the daily intake [of a chemical] which, during an entire lifetime, appears to be without appreciable risk on the basis of all the known facts of the time. For this purpose 'without appreciable risk' is taken to mean the practical certainty that injury will not result even after a lifetime of exposure..." Of the ten chemicals listed, eight are organochlorine compounds, one is a carbamate, and one an organophosphate. Chlordanes are noteworthy by their absence. Even heptachlor, from all sources -- the least of which is technical chlordanes -- occurs as a maximum of 10% of the ADI in the period of 1965-66; the trend has been downward to a level of 4% of the ADI in 1970.

I understand that Canadian Total Diet Studies yield comparable findings.

After more than two decades of use, it has become apparent that both chlordanes and heptachlor have their greatest

benefit to risk advantage when used as soil insecticides (except for foliar application of chlordane on cotton). In agriculture, chlordane and heptachlor are applied to soil before planting and afford protection to crops from soil insects without significant hazard to the environment nor to humans. Both compounds are degraded under the influence of soil micro-flora and fauna at a rate corresponding to a half-life of about 1 year, a rate which provides good pest control at one application per season.

Both compounds have also been used at much higher rates, applied locally, for termite control of structures. Because the rate of decline of activity is considerably slower at the high concentration levels applied for termite control, single applications have given protection in excess of 20 years. Especially noteworthy is the fact that under both agricultural and termite-control conditions, neither chlordane nor heptachlor applied to soil is mobile. Hence, they afford protection to the area of application without invading the surrounding areas. This property minimizes environmentally adverse effects.

LIST OF EXHIBITS

- EXHIBIT I - Bevenue, A. and Yeo, C. Y., 1969. Gas Chromatographic Characteristics of Chlordane - II. Observed Compositional Changes of the Pesticide in Aqueous and Non-Aqueous Environments. J. Chromatog., 42: 45-52.
- EXHIBIT II - Edwards, C. A., 1970. Persistent Pesticides in the Environment. Critical Reviews in Environ. Control, 1 (1): 7-67. (Excerpt Tables 12, 13, 15: Pages 29, 31, 34.)
- EXHIBIT III - Polen, P. B., Hester, M. and Benziger, J., 1970. Characterization of Oxychlordane, Animal Metabolite of Chlordane. Bull. Environ. Contam. Toxic., 5 (6): 521-528.

LIST OF EXHIBITS - (Continued)

- EXHIBIT IV - Council on Environmental Quality, 1971.
Environmental Quality, Second Annual Report.
(Table A-6, Page 248).
- EXHIBIT V - Boyd. J. C., 1970. Field Study of a Chlordane
Residue Problem in Milk. Bull. Environ.
Contam. Toxic., 5 (4): 292-299.
- EXHIBIT VI - Reports of IUPAC Commission on Terminal
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- (d) Hill, K., 1970. Ibid., 53 (5): 991-2.
- (e) Hill, K., 1971. Ibid., 54 (6): 1318-1322.
- EXHIBIT VII - Joint Meeting of Pesticide Residue Experts of
FAO/WHO 1971. Excerpts on "Metabolism of
Chlordane in Mammals" and "Metabolism of
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CHROM. 4052

GAS CHROMATOGRAPHIC CHARACTERISTICS OF CHLORDANE

II. OBSERVED COMPOSITIONAL CHANGES OF THE PESTICIDE IN AQUEOUS AND NON-AQUEOUS ENVIRONMENTS*

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SUMMARY

Studies were made on the vaporization and adsorptive properties of chlordane when the pesticide was exposed to water and organic solvent environments. Within thirty days of exposure to water, the heptachlor component of chlordane was completely changed to 1-hydroxychlordane. Over a period of sixty days, water adsorbed increasing amounts of vaporized chlordane; the α -chlordane, γ -chlordane and nonachlor components of the pesticide were markedly stable. No apparent chemical changes in the pesticide were observed when it was exposed to an isooctane environment for an equivalent period of time; the more volatile components of chlordane were adsorbed to a greater degree in the organic solvent.

Hexachlorocyclopentadiene, a component of chlordane, dissipated or degraded, with time, in water solution. However, in an organic solvent environment, this chemical displayed multi-component characteristics.

Gas chromatography was used to observe the changes in the characteristics of the pesticide, with time, in aqueous and non-aqueous media.

INTRODUCTION

Data were acquired by gas chromatography on some of the apparent chemical changes and vaporization and adsorption properties of the pesticide chlordane (a mixture of octachloro-4,7-methanotetrahydroindane and related compounds including hexachlorocyclopentadiene) in organic solvent and aqueous media, which were considered necessary prerequisites to a study of the adsorptive effects of this chemical on stored foodstuffs. Comparable data were also acquired on hexachlorocyclopentadiene which may exist in an amount as large as 1% in commercially available chlordane.

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MATERIALS AND METHODS

Gas chromatograph

F & M Model 810, electron capture detector; column $\frac{1}{8}$ in. \times 4 ft. borosilicate glass containing 3% SE-30 silicone on Chromosorb W, 80-100 mesh, acid-washed and treated with dimethyldichlorosilane; column temperature 190°, injection temperature 200°, detector temperature 200°, argon-methane (90-10) carrier gas, flow rate 75 ml/min; Leeds and Northrup Speedomax H recorder, 1 mV full scale, chart speed $\frac{1}{2}$ in. per min.

Reagents

Hexane and isooctane, Mallinckrodt Nanograde.

Pesticide chemicals

Chlordane, technical grade; α -chlordane, γ -chlordane, heptachlor, heptachlor epoxide (the foregoing chemicals supplied through the courtesy of the Velsicol Corp., Chicago, Ill.), chlordene and nonachlor. 1-Hydroxychlordene was supplied through the courtesy of Dr. M. C. BOWMAN, USDA, ARS, Tifton, Ga.

Hexachlorocyclopentadiene (Aldrich Chemical Co., Milwaukee, Wisc.). All of the pesticide chemicals were 99.4 to 99.8% purity with the exceptions of technical grade chlordane and hexachlorocyclopentadiene.

Experimental

(a) A glass vial containing 50 mg technical grade chlordane was placed in each of 15 8-oz. glass jars (60 mm diameter) which contained 20 ml isooctane; the vial extended above the solvent surface to permit only the vaporized components of the chlordane to contact the solvent. The jars were sealed with aluminum foil including a bleed hole to allow equilibration with the atmosphere. The jars were selected at 2-day intervals over a period of 30 days for analysis of the isooctane contents to determine the characteristics of the gas chromatographic curve of the vaporized chlordane components adsorbed by the solvent. Another set of jars was prepared, replacing the isooctane with 20 ml distilled water, for similar analysis of the water for adsorbed chlordane vapors.

(b) Two series of jars were prepared (one of isooctane and one of distilled water, 20 ml of each solvent) as described in (a), but 0.5 mg hexachlorocyclopentadiene (Hex) was substituted for the chlordane component. The amount of Hex selected was considered to be relative to the maximum amount that was present in the technical chlordane¹ used in (a).

(c) Twenty milliliters of distilled water containing 1 p.p.m. (20 μ g) technical grade chlordane was added to each of twelve 50-ml glass-stoppered erlenmeyer flasks and stored. Flasks were selected at 2-day intervals over a period of 30 days for chlordane analysis. The contents of the twelfth flask were analyzed after 60 days of storage.

All of the samples were stored at room temperature (22-25°) and a relative humidity of 60-80%. No direct sunlight contacted the samples, and fluorescent light was the primary light source in the laboratory in which these experiments were conducted.

Preparatory to gas chromatographic analysis, the chlordane-isooctane solutions

were transferred to 50-ml volumetric flasks and made to volume with isooctane. The Hex-isooctane solutions were made to 100-ml volumes. The water solutions were extracted three times with hexane, the combined hexane extracts were filtered through a plug of anhydrous sodium sulfate directly into 50-ml volumetric flasks and made to volume with hexane. Suitable aliquots were applied to the gas chromatograph and the recorded data were compared to data obtained from the technical chlordane and related chemical standards.

RESULTS AND DISCUSSION

Technical grade chlordane, described by the manufacturer to be composed of a mixture of octachloro-4,7-methanotetrahydroindane and related compounds with a maximum of 1% hexachlorocyclopentadiene¹, contains at least ten components² including heptachlor and γ - and α -chlordane (see Fig. 1, curve 1). Although chlordane is a complex mixture of chlorinated hydrocarbons, the product is surprisingly uniform in composition as determined by acceptable methods of quality control³.

Gas chromatograph curves of chlordane have appeared only recently in the literature^{2,4-7}, which is not surprising considering the complex pattern of the chromatographed material. KAWAHARA *et al.*⁸ presented a study of gas chromatograph retention times of some chlordane components but they did not include any illustrative curves. The number of characteristic gas chromatograph peaks of chlordane obtained by different workers have varied from seven⁶ to fourteen²; such differences were due, no doubt, to variable gas chromatograph techniques and not to any characteristic differences in the chlordane material used by each worker. In all instances, including the data reported herein, the predominant peak areas (Fig. 1, curve 1) were heptachlor (E), the peak preceding heptachlor (D), and γ -chlordane and α -chlordane (J, K).

Fig. 1 illustrates the adsorption pattern of vaporized technical chlordane exposed to isooctane for time periods of 2 to 30 days. The greater volatility of the heptachlor component (peak E) of chlordane and the fractions that emerged from the gas chromatograph column prior to heptachlor are readily apparent. The gas chromatograph curve pattern of technical chlordane in isooctane solution was unchanged over a period of 30 days stored at room temperature (20–25°) with exposure to artificial light, except that the more volatile components of the pesticide became magnified by continued adsorption of these components by isooctane. This stability characteristic is in agreement with BURKE AND HOOVER⁹ who noted that other chlorinated pesticides, including heptachlor and heptachlor epoxide, exhibited no characteristic changes in composition over a period of 8 months when isooctane solutions of the pesticides were stored under similar room conditions.

The data obtained from isooctane solutions of the adsorbed vapors from Hex showed an unexpected gas chromatograph pattern. After 24 h of exposure to isooctane, the chromatographic characteristics of Hex were similar to the standard material obtained directly from the reagent bottle (Fig. 2, curves 2 and 3). However, the curves 4, 5, and 6 (Fig. 2) for the solutions exposed 7 through 21 days showed a multiple-peak phenomena up to and including the D-peak area of technical chlordane; this latter peak area (D) has been described as the Diels-Alder adduct of pentachlorocyclopentadiene and cyclopentadiene². The degree of purity of hexachlorocyclopentadiene was not determined; however, the source of this reagent (Aldrich Chemical Co.)

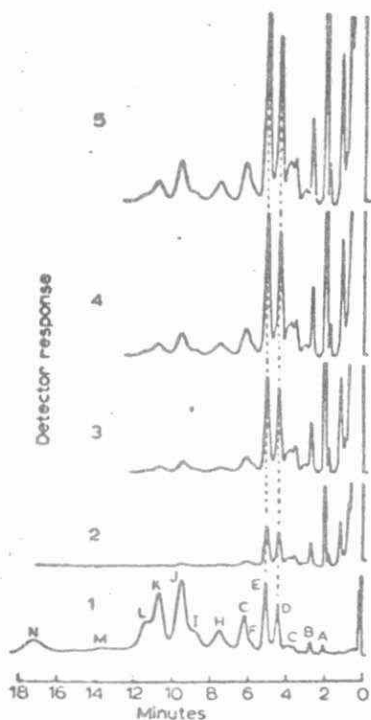


Fig. 1. Technical chlordane vapors exposed to isooctane. Curve 1, 4 ng technical grade chlordane standard; curve 2, 2 days exposure; curve 3, 10 days exposure; curve 4, 20 days exposure; curve 5, 30 days exposure.

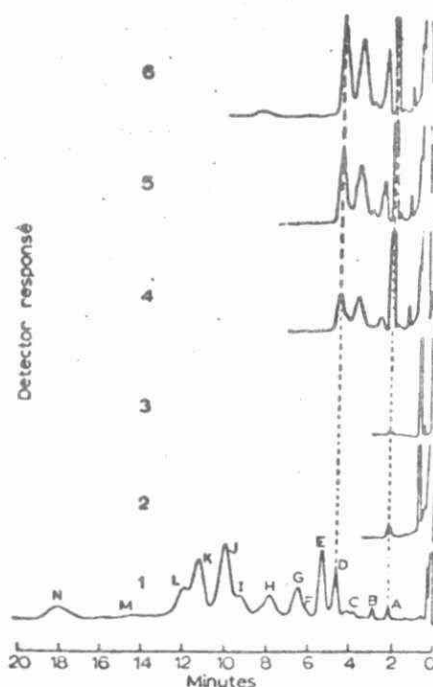


Fig. 2. Hexachlorocyclopentadiene vapors exposed to isooctane. Curve 1, 4 ng technical grade chlordane standard; curve 2, 0.1 ng hexachlorocyclopentadiene standard; curve 3, 1 day exposure; curve 4, 7 days exposure; curve 5, 14 days exposure; curve 6, 21 days exposure.

indicates a purity range of 97–100%. Also, the method of manufacture of this chemical will largely determine the number of other chlorinated hydrocarbons that may be present in the final product¹⁰. Some of the early peaks observed in isooctane solution containing adsorbed technical chlordane vapors (Fig. 1) may be attributed to the Hex fraction of the pesticide, as illustrated in Fig. 3. Fig. 4, curve 3 shows another comparison of the adsorbed Hex vapors with known standards of possible chlordane components (curve 2), and indicates that either chlordene, an intermediate product formed during the manufacture of chlordane¹¹, may be a constituent of the Hex pattern and/or the components shown in the early part of the curve may be more susceptible to atmospheric oxidation and/or light, as suggested by BROOKS AND HARRISON's studies with chlordene¹². The data suggest that any examination of foodstuffs (especially those of low moisture content) which contain adsorbed chlordane residues would yield gas chromatograph data containing a multiple-peak picture prior to the D area similar to curves 2 and 3, Fig. 3, instead of a pattern as in curve 1, Fig. 3.

The data obtained from distilled water solutions containing adsorbed vapors of Hex was different from the comparable data for isooctane solutions. After 3 days exposure, any adsorbed Hex components had disappeared completely from the water

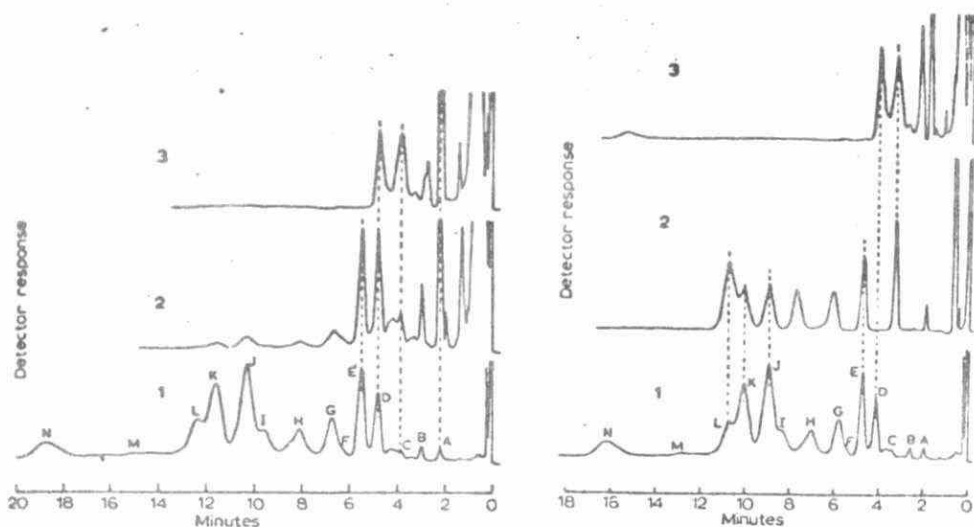


Fig. 3. Curve 1, 4 ng technical grade chlordane standard. Vapors exposed to isooctane for 14 days; curve 2, technical grade chlordane; curve 3, hexachlorocyclopentadiene.

Fig. 4. Curve 1, 4 ng technical grade chlordane standard. Curve 2, standards including possible chlordane components—the seven peaks reading from left to right on the curve, nonachlor, α -chlordane, γ -chlordane, heptachlor epoxide, 1-hydroxychlordene, heptachlor, chlordene. Curve 3, hexachlorocyclopentadiene vapors exposed to isooctane for 14 days.

(Fig. 5), suggesting dissipation or decomposition of the chemical. KAWAHARA *et al.*⁸ noted that Hex decomposed rapidly upon exposure to light or during the period of analysis of the chemical by thin layer chromatography.

Data obtained from distilled water samples which had been exposed to the vapors of technical chlordane are illustrated in Fig. 6. The noncumulative effect of the more volatile components of the pesticide is consonant with the data obtained with Hex and water. Over a period of 60 days, the water adsorbed increased amounts of the vaporized chlordane with no change in the characteristics of the gas chromatograph curves between the areas of peaks H and L, indicating a marked stability to water and light of the γ -chlordane, α -chlordane, and nonachlor components of the pesticide. A tangible difference in the gas chromatograph characteristics of the area of the curve

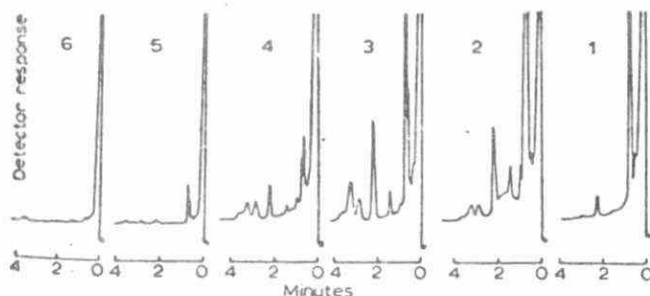


Fig. 5. Hexachlorocyclopentadiene vapors exposed to distilled water. Curve 1, 1.0 ng standard solution; curve 2, 1 day exposure; curve 3, 2 days exposure; curve 4, 3 days exposure; curve 5, 5 days exposure; curve 6, 14 days exposure.

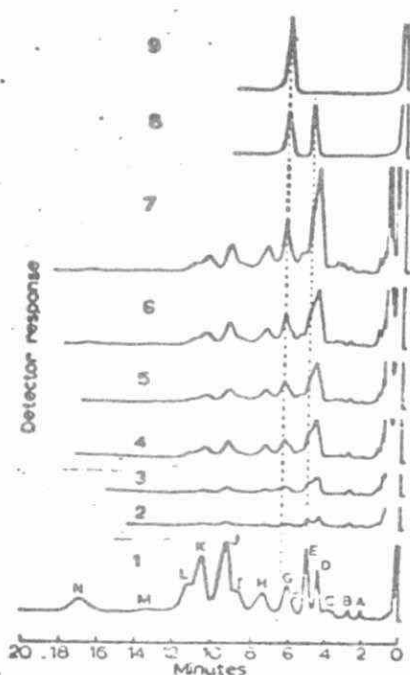


Fig. 6. Technical grade chlordane vapors exposed to distilled water. Curve 1, technical grade chlordane standard, 4 ng; curve 2, 1 day exposure; curve 3, 7 days exposure; curve 4, 14 days exposure; curve 5, 21 days exposure; curve 6, 30 days exposure; curve 7, 60 days exposure; curve 8, heptachlor vapors exposed to distilled water for 14 days; curve 9, 1-hydroxychlordene (1 p.p.m.) in water, 14 days storage.

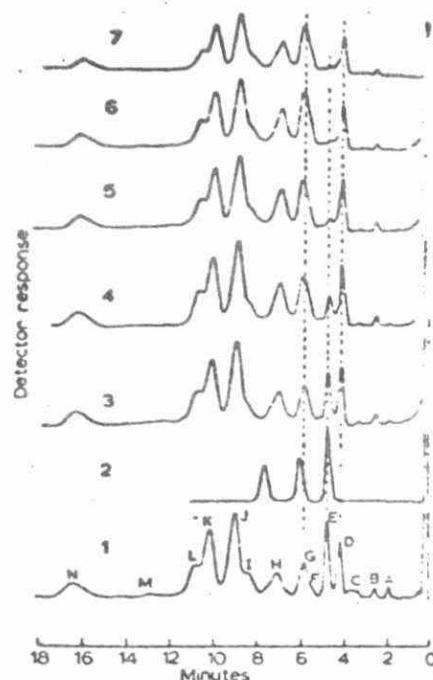


Fig. 7. Changes in characteristics of technical grade chlordane, with time, stored at a concentration of 1 p.p.m. in distilled water. Curve 1, technical grade chlordane standard, 4 ng; curve 2, heptachlor epoxide, 1-hydroxychlordene, heptachlor standards, reading from left to right, each 0.2 ng; curve 3, 4 days storage; curve 4, 12 days storage; curve 5, 20 days storage; curve 6, 30 days storage; curve 7, 60 days storage.

between peaks D and G (Fig. 6) was apparent within 7 days of exposure and the change was quite marked between the period of 21 days and 60 days of exposure. In addition to the heptachlor component of the pesticide converting to 1-hydroxychlordene, similar to an observation noted by BOWMAN *et al.*^{13,14}, the D peak area characteristic was tangibly changed. There was no evidence of the formation of heptachlor epoxide. COCHRANE AND CHAU¹⁵ recommended the conversion of 1-hydroxychlordene to its corresponding silyl ether derivative to produce a more sensitive and sharper peak on their gas chromatograph curves; this additional step was not considered necessary (see Fig. 6, curve 9), using the gas chromatograph conditions reported herein.

The storage pattern of technical chlordane in water solution (1 p.p.m.) over a period of 60 days showed somewhat different gas chromatograph characteristics (Fig. 7) than the water which was exposed to the vapors of chlordane. The gradual loss of the heptachlor component (peak E) was more apparent, and almost complete loss of this component occurred after 16 days of storage; loss was complete after 30 days of storage. Changes in the profile of peak G of the series in Fig. 7 became quite marked after 16 days of storage because of the formation of 1-hydroxychlordene which was

TABLE I

CHANGE IN CONCENTRATION OF TECHNICAL CHLORDANE IN WATER SOLUTION

Age of solution (days)	Curve areas*				
	Peak H	Peak I J	Peak K L	Total	Peak G-OH
2	0.20	0.64	0.65	1.49	0.20
4	0.25	0.61	0.64	1.50	0.24
6	0.26	0.70	0.69	1.65	0.30
8	0.23	0.56	0.60	1.39	0.30
10	0.25	0.70	0.70	1.65	0.30
12	0.30	0.65	0.64	1.59	0.35
14	0.27	0.60	0.60	1.47	0.30
16	0.26	0.53	0.50	1.29	0.30
18	0.27	0.53	0.58	1.43	0.30
20	0.28	0.56	0.55	1.39	0.30
30	0.27	0.53	0.53	1.33	0.30
60	0.20	0.43	0.40	1.03	0.26

* Areas of designated peaks of curves illustrated in Fig. 7, measured by planimeter in square inches. Each curve represents an original quantity (at zero time) of 2 ng technical chlordane.

practically superimposable on the component G of the original technical chlordane. Experience has shown that some evaporation loss may occur with glass-stoppered flasks, and the data in Fig. 8 and Table I indicate some codistillation of the pesticide^{14,16}. However, in the area of peak G of the curve, this factor is not apparent, because of the increased superimposition of 1-hydroxychlordane, with time, that was formed from the degraded heptachlor component. Supplemental storage studies of 1-hydroxychlordane in water after 2, 7 and 14 days indicated no change in the gas chromatograph characteristics of the compound and there was no apparent loss of this component by codistillation. In this series, also, no heptachlor epoxide was formed during the storage period.

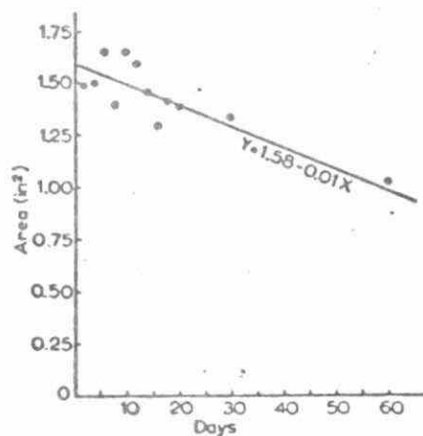


Fig. 8. Codistillation of technical grade chlordane with water (see Table I).

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SOURCE: "Persistent Pesticides in the Environment" by Clive A. Edwards, Critical Reviews in Environmental Control, 1 (1) 7-67 (1970).

EXHIBIT II

TABLE 12

CONCENTRATION OF RESIDUES FROM WATER INTO AQUATIC INVERTEBRATES

Site	Reference	Organism	Insecticide	Amount		Concentration factor**
				In water (pp 10 ⁻⁹) ^a	In animals (pp 10 ⁻⁶) [†]	
U.S.A.	Terriere et al. 1965 (250)	Aquatic invertebrates	Toxaphene	0.63	1.43	2,270
U.S.A.	Butler 1965 (32)	Shrimp	DDT	0.5	0.14	2,800
U.S.A.	Cope 1966 (40)	Crayfish	DDT	20.0	1.47	73
U.S.A.	Bridges et al. 1963 (24)	Crayfish	DDT	20.0	2.0	100
U.S.A.	Cole et al. 1967 (44)	Crayfish	DDT	24.0	2.32	97
U.S.A.	U.S.D.I. 1963 (262)	Crayfish	DDT	20.0	0.33	16.5
U.S.A.	Hunt & Bischoff 1960 (135)	Plankton	DDT	20.0	5.0	250
U.S.A.	Keith 1964 (151)	Plankton	DDT	0.3	5.0	16,666
U.S.A.	U.S.D.I. 1964 (263)	Eastern oyster	DDT	10.0	151.0	15,100
		Eastern oyster	DDT	1.0	30.0	30,000
		Eastern oyster	DDT	0.1	7.0	70,000
		Pacific oyster	DDT	1.0	20.0	20,000
		Hard clam	DDT	1.0	3.0-	3,000-
					9.0	9,000
		Eastern oyster	Dieldrin	1.0	3.5	3,500
		Commercial shrimp	DDT	0.5	0.14	250
		Sea squirt	DDT	100.0	20.0	200
				10.0	10.0	1,000
		Sea squirt	DDT	0.1	20.0	200,000
		Sea squirt	DDT	0.01	10.0	1,000,000
		Sea hare	DDT	0.01	1.78	178,000
		Crabs	DDT	50.0	7.2	144
		Snails	DDT	50.0	74.0	1,480
U.S.A.	Bugg et al. 1967 (29)	Oysters	BHC	0.33	0.006	18.2
			DDT	0.55	0.033	60.0
			Dieldrin	0.44	0.006	13.6
			Heptachlor	0.55	0.002	3.6
U.S.A.	Godsil & Johnson 1968 (103)	Clams	DDT	5.8	0.008	1.4
			Chlordane	6.6	0.006	0.9
U.S.A.	Loosanoff 1965 (177)	Common clam	Endrin	10.5	0.013	1.2
U.S.A.		Oyster	DDT	0.1	7.0	70,000
U.S.A.	Butler 1966 (33)	Hocked mussel	Dieldrin	1.0	3.5	3,500
		Eastern oyster	DDT	1.0	24.0	24,000
		Pacific oyster	DDT	1.0	26.0	26,000
		European oyster	DDT	1.0	20.0	20,000
		Crested oyster	DDT	1.0	15.0	15,000
		Northern quahogs	DDT	1.0	23.0	23,000
			DDT	1.0	6.0	6,000

^a µg/litre

[†] mg/kg

** Concentration factor = $\frac{\text{concentration in animal}}{\text{concentration in water}}$

TABLE 13
CONCENTRATION OF RESIDUES FROM WATER TO FISH

Organism	Insecticide	Amount of residue		Concentration factor**	Reference
		In water (pp 10 ⁻⁹)*	In animal (pp 10 ⁻⁶)*		
Rainbow trout	DDT	20.0	4.15	207	Cope 1966 (46)
Black bullhead	DDT	20.0	3.11	155	"
Bluegill	Heptachlor	50.0	15.7	314	"
Catfish	Aldrin & dieldrin	0.044	0.07	1,590	Sparr et al. 1966 (236)
"	"	0.009	0.04	4,444	"
"	"	0.021	0.02	952	"
"	"	0.007	0.01	1,428	"
Buffabfish	"	0.023	0.09	3,913	"
"	"	0.007	0.21	30,000	"
Scaled sardine	DDT	0.1	0.11	1,100	Butler 1965 (32)
Rainbow trout	Toxaphene	0.41	7.72	18,829	Terriere et al. 1965 (250)
Fish	DDT	0.30	1.0 - 6.4	3,333 - 21,333	Keith 1964 (151)
"	Endrin	0.10	7.0	70,000	Larger 1964 (163)
"	Toxaphene	1.0 - 4.0	0.8 - 2.5	2,000 - 2,500	Kailman et al. 1962 (149)
" (5 spp.)	DDT	30 - 40	4 - 58	130 - 1,450	Crocker & Wilson 1965 (51)
Bullhead trout	DDT	20	2 - 4	100 - 200	Bridges et al. 1963 (24)
Fathead minnows	Endrin	0.015	0.15	10,000	Mount & Putnicki 1966 (193)
Croakers	DDT	0.1	2.0	20,000	Hansen 1966 (110)
Pinfish	DDT	1.0	12.0	12,000	"
"	DDT	0.1	4.0	40,000	"
Fish	Dieldrin & DDT	10.0	0.1 - 1.0	10 - 100	Holden & Maraden 1966 (131)
Trout	Dieldrin	2.3	7.7	3,300	Hollen 1966 (130)
Chubs	DDT	5.8	0.029	5	Godsil & Johnson 1968 (103)
"	Chlordane	6.6	0.003	1.2	"
"	Endrin	10.5	0.050	4.7	"
Trout	DDT	20.0	4.0	200.0	U.S.D.I. 1963 (262)
Bluegills	Heptachlor	50.0	56.8	1,130.0	U.S.D.I. 1964 (263)
Fish	DDT	0.015	12.44	829,300	Mack et al. 1964 (181)
"	DDT	0.11	3.85	35,000	"
White catfish	DDD	14.0	30.4 - 129.0	2,172 - 9,214	Hunt & Bischoff 1960 (135)
Large-mouth bass	DDD	14.0	19.7 - 25.0	1,407 - 1,705	"
Brown bullhead	DDD	14.0	15.5 - 24.8	1,107 - 1,771	"
Black crappie	DDD	14.0	5.4 - 115.0	356 - 8,214	"
Bluegill	DDD	14.0	6.6 - 10.0	471 - 714	"
Sacramento blackfish	DDD	14.0	10.9 - 17.6	778 - 1,257	"
Brook trout	DDT	24.0	17.3	710.0	Cole et al. 1967 (44)

* µg/liter

* mg/kg

** Concentration factor = $\frac{\text{Concentration in animal}}{\text{Concentration in water}}$

TABLE 15

MOVEMENT OF INSECTICIDES FROM SOIL OR WATER INTO PLANTS

Insecticide	Crop	Source of insecticide	Residues			Reference
			Amount in source (pp 10 ⁻⁹)*	Amount in plant (pp 10 ⁻⁶)†	Concentration or dilution factor**	
Toxaphene	Aquatic plants	Water	0.41	0.21	512.0	Terriere et al. 1965 (250)
DDT	Aquatic vegetation	Water	200.0	75.0	375.0	Crocker & Wilson 1965 (51)
DDT	"	Water	20.0	31.0	1,550.0	Bridges et al. 1963 (24)
Organo-chlorines	Aquatic plants	Water	0.45	1.0	2,220.0	Keith 1966 (152)
Organo-chlorines	"	Water	0.35	1.1	3,171.0	"
Organo-chlorines	"	Water	0.23	0.8	3,478.0	"
Organo-chlorines	"	Water	0.30	30.3	100,000.0	"
DDT	Algae & moss	Water	0.33	0.01	33.0	Mack et al. 1964 (181)
DDT	Algae	Water	5.8	0.002	0.34	Godsil & Johnson 1968 (103)
Chlordane	"	Water	6.6	0.013	1.97	"
Endrin	"	Water	10.5	0.007	0.66	"
DDT	Vascular plants	Water	5.8	0.003	0.52	"
Chlordane	"	Water	6.6	0.003	0.45	"
Endrin	"	Water	10.5	0.006	0.57	"
			(pp 10 ⁻⁶)†	(pp 10 ⁻⁶)†		
Aldrin	Carrot roots	Muck soil	6.36	0.01(root)	0.0013	Hartig & Harris 1966 (141)
Aldrin	"	Clay soil	0.48	0.01 "	0.021	"
Dieldrin	"	Muck soil	3.9	0.02	0.0051	"
Dieldrin	"	Clay soil	0.48	0.11	0.23	"
Heptachlor	Rutabagas roots	Soil	0.320	0.024	0.075	Saha & Stewart 1967 (232)
Heptachlor	Wheat foliage	Seed treated	543.0	0.015	0.036	Burrage & Saha 1967 (30)
Aldrin & dieldrin	"	Soil	1.8	0.014	0.0077	Saha & McDonald 1967 (231)
Aldrin & dieldrin	Cucumber fruit	Soil	3.7	0.113	0.031	Lichtenstein & Schulz 1965 (174)
Heptachlor	"	Soil	3.8	0.091	0.024	"
Dieldrin	"	Soil	1.4	0.043	0.031	"
Aldrin	Alfalfa	Soil	0.84	0.009	0.011	"
Heptachlor	"	Soil	0.78	0.028	0.036	"
Aldrin	Carrot roots	Soil	0.94	0.32	0.34	Lichtenstein et al. 1968 (176)
Aldrin	Potato tuber	Soil	0.94	0.07	0.074	"
Heptachlor	Carrot roots	Soil	0.49	0.36	0.73	"
Heptachlor	Potato tuber	Soil	0.49	0.05	0.10	"
DDT	Alfalfa foliage	Soil	1.39	0.113	0.08	Ware et al. 1968 (274)

Characterization of Oxychlordanes, Animal Metabolite of Chlordane*

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Recognition that α - and γ -chlordane (CHL) comprise the principal terminal residues on plants treated with Technical CHL (1) prompted study of these isomers, the main components of Technical CHL -- as distinguished from the total composition. A surprising observation was made as a by-product of one toxicological study wherein massive doses of pure CHL isomers were fed to rats. It appeared from gas-liquid chromatographic analysis of the rat fat that heptachlor epoxide (HE) had been formed as a metabolite (2). The present study shows this conclusion to be an artifact resulting from the limitations of a widely used chromatographic column (DC-200) and that a heretofore unrecognized compound resulted from the metabolism of α - or γ -CHL.

Critical study disclosed means of distinguishing the newly recognized metabolite from HE and provided tools for study of a new aspect of CHL metabolism.

A source of the metabolite for suitable isolation was found in the fat of pigs ingesting for 90 days diets fortified with massive doses (300 ppm) of individual CHL isomers (3). Pure metabolites isolated from feeding of either α - or γ -CHL were compared and found to be identical by melting point, electron capture gas-liquid chromatography, thin-layer chromatography, infrared and nuclear magnetic resonance spectroscopy and p-values. The metabolite, now called oxychlordanes (OXY), $C_{10}H_4Cl_8O$, appears to form from CHL in the reaction $C_{10}H_8Cl_8 + 2(O) \longrightarrow C_{10}H_4Cl_8O + H_2O$.

OXY has also been synthesized in vitro by oxidation of 1,2-dichlorochlordene-2 and through direct oxidation of the respective CHL isomers with chromic acid (3).

While the pure isolated metabolite from feeding separately two isomers of CHL and the synthetic compound are analytically equivalent, at least one of the in vivo isolates is optically active. Limited bio-assays with house flies may indicate that the synthetic compound is about double the potency of the isolates. This observation coupled with optical activity of one of the isolates indicates selective metabolism of one

*Results of research suggested by Commission on Terminal Pesticide Residues, International Union of Pure and Applied Chemistry (IUPAC).

of the enantiomorphs by animals. Similar selective enantiomorphic enrichment has been reported in the metabolism of dieldrin in rabbits (4) and microsomal conversion of other cyclodienes (5).

ANALYTICAL CHARACTERIZATION OF OXYCHLORDANE (OXY)

Melting Point. α -isolate, 99.4-100.0°C.; γ -isolate, 99.0-101.0°C.; synthetic, 99.0-101.0°C. (all uncorrected).

Elemental Analysis. Theory for $C_{10}H_4Cl_8O$: 28.34% Cl, 0.95% H, 66.93% Cl, 3.78% O. Found: γ -isolate - 28.42% Cl, 1.00% H, 66.92% Cl, 3.66% O, (by difference); α -isolate - 66.83% Cl.

Nuclear Magnetic Resonance Spectrum (NMR). α -, γ -isolates and synthetic OXY exhibited the same NMR spectra showing a multiplet at 3.20 to 3.65 ppm (2H; H-C-C-H); a singlet, 3.85 ppm (1H; epoxy); and doublet, 4.30 to 4.40 ppm (1H; H-C-C-H).

Infrared Absorption Spectrum (IR). α -, γ -isolates and synthetic OXY exhibited identical infrared absorption spectra (Fig. 1).

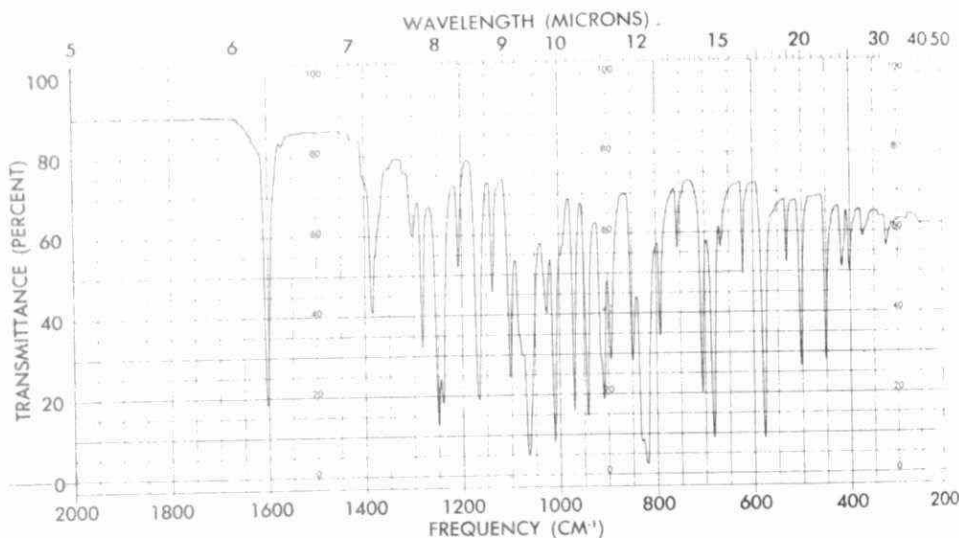


Figure 1. IR Spectrum of OXY in KBr Pellet

Optical Rotation. OXY isolated from γ -CHL feeding of pigs, $(\alpha)_D^{25} + 2.7$ deg. dec. $^{-1}g.^{-1}ml.$; α -CHL feeding isolate and synthetic product, no optical rotation observed.

Gas Liquid Chromatography (GLC). Some columns fairly widely used for GLC analysis of organochlorine pesticides do not resolve OXY from HE. Clues to the presence of OXY with HE are broader peaks and slight discrepancy of RT compared with pure HE standards. For example, on DC-200/Epon 1001 columns the broadened, unresolved peaks for OXY-HE mixtures have retention time (RT) 0.92-0.98 relative to HE standards, depending on ratio of constituents.

Good resolution into 2 peaks is observed with either EC or micro-coulometric detectors and OV-17/QF-1 (various ratios) columns at 180° - $225^{\circ}C.$: RT for OXY relative to HE -- 0.87-0.90. (RT for OXY relative to aldrin is 1.30-1.35). The RT for OXY on a 4-foot, 3% QF-1, 80/100 mesh Gas Chrom Q column at 165° with flame ionization detector is 0.82 relative to HE.

p-Values. Measured according to Beroza and Bowman (6), observed p-values are: In heptane/ CH_3CN , 0.44-0.49 for OXY, 0.26-0.28 for HE; in heptane/85% aqueous dimethyl formamide, 0.70-0.78 for OXY, 0.44-0.55 for HE.

Thin layer Chromatography (TLC). Rf's observed on EK 100μ plates for 95% heptane, 5% acetone solvent system are for OXY and HE respectively: Alumina 0.54, 0.40; Silica Gel 0.41, 0.34.

Response to Acid and Alkali. Stable to acid. No appreciable change in GLC peak height upon 18 hour contact of a $0.1\mu g.$ OXY per ml. n-heptane solution with H_2SO_4 /fuming H_2SO_4 (1:1).

Unstable to alkali. No visible GLC peaks from direct pentane extract of reaction mixture: 20% ethanolic KOH at $55^{\circ}C.$ for 30 min.; H_2O added. Extraction of same mixture, after acidification with H_2SO_4 , yielded 3 peaks at 7.5, 10.2, and 12.0 minutes. The RT for OXY under identical conditions is 4.7 minutes.

Response to Chromogenic Reagents. OXY treated with Polen-Silverman (7, 8) or Davidow (9) reagents produces yellow solutions, visually of the same hue as those from HE but less intense. These reagents provide no self-revealing qualitative distinction between OXY and HE.

STORAGE OF OXY IN FAT OF MAMMALS

Rats. White rats, which for one year ingested diets fortified with either the α - or γ -CHL isomers of 50-50 mixtures, formed and stored OXY in their fat at levels shown in Table 1. The ratio of OXY concentration found in fat to that of CHL consumed is given as Storage Concentration Ratio.

To aid in assessing these observations it is interesting to note that the "no effect" level for CHL in rats is estimated to be 20 ppm in the diet, equivalent to 1 mg/kg/day (5).

TABLE 1

OXY Storage Levels in Fat of Rats*
Fed One Year on Diets Dosed with
Chlordane Isomers

Isomer	PPM in Diet (D)	OXY in Fat, PPM - (θ)**	Storage Conc. Ratio, θ/D
Control	0	0.2	--
α	5	8.	1.6
"	15	7.	0.5
"	15	13.	0.9
"	15	12.	0.8
"	45	22.	0.4
γ	75	72.	1.0
"	75	93.	1.2
"	75	105.	1.4
"	150	150.	1.0
50-50 α & γ	45	55.	1.1
"	75	75.	1.0

*Both sexes included. Each line represents one individual, but identity of sex was not annotated.

**Calibrated against HE standard; not corrected for value observed for control.

Dogs. Beagle dogs continually consuming diet spiked with Technical CHL stored, after two years, OXY at levels as shown in Table 2. The fat also gave chromatographic responses related to other constituents of Technical CHL.

The evaluation of these results may be aided by noting that the "no effect" level for CHL in dogs has been estimated to be 3.0 ppm in the diet, equivalent to 0.075 mg/kg/day (5).

TABLE 2

OXY Storage Levels in Fat of Dogs
Fed 2 Years on Diet Dosed with
Technical Chlordane

CHL Fed, ppm -- (F)	Sex	OXY in Fat, ppm -- (θ)*	Storage Conc. Ratio -- (θ/F)
3 ppm	M	3.0	1.0
3 ppm	F	3.7	1.2
30 ppm	M	24.	0.8

*Analyses are made on fat of individual animals.

Figs. Pigs administered high levels of CHL isomers in their diets for 90 days provided an adequate source for isolating the metabolite. Six pigs were fed diets fortified at 300 ppm (equivalent to 3-9 mg/kg, decreasing as body weight increased) with individual CHL isomers. No visible gross side reactions were observed in any of the experimental subjects. Analyses of fat in animals sacrificed at 60 and 90 days are given in Table 3. The fat of these animals provided the source for isolation of pure metabolite (3) which was subsequently determined to be OXY formed from both CHL isomers.

TABLE 3

Residue Storage Levels in Fat of Pigs
On Feed Dosed at 300 ppm with Chlordane Isomers

Isomer	Days	Found in Fat, ppm		Storage Conc. Ratio	
		OXY (θ)*	CHL Isomer Fed (C)	OXY (θ/300)	CHL (C/300)
α π	60	12.	8.	0.03	0.027
	90	36.	9.	0.12	0.030
γ π	60	69.	9.	0.23	0.030
	90	71.	4.	0.24	0.013

*Calibrated against HE standard.

Briefly, isolation of the metabolite resulted from successive partitions between hexane and acetonitrile, acid and chromatographic clean-up, and recrystallization from pentane. Experimental details and proposal of structure for OXY are given elsewhere (3).

Assuming 20% fat in pigs, an estimate of the fraction of ingested CHL isomers stored as residues is given in Table 4. The magnitude is substantially in agreement with the findings of Korte (10) that CHL is largely dissipated in urine and feces as hydrophilic metabo-

lites; he estimated storage in fat of rabbits at about 4% of γ -CHL (called α -CHL in his nomenclature) consumed for 10 weeks. Our observations indicate that the formation and storage of metabolite is smaller for α -isomer than for γ -CHL.

TABLE 4

Propensity for Residue Storage
As Percent of CHL Consumed (Pigs)

90 Days, 300 ppm CHL in Diet					
Isomer	CHL in Diet mg.	Stored in Fat, mg.		Stored, as % of CHL Fed	
		OXY	CHL	OXY	CHL
α	83,100	770	190	0.9	0.2
γ	82,200	1780	100	2.2	0.1

Cows (Beef and Dairy). To learn if OXY occurs in meat or milk as a result of feeding CHL at tolerance level proposed by FAO/WHO (11), 4 beef cattle and 12 dairy cattle were given feed dosed with pure chlordane isomers. Pure α -, γ -CHL and 50-50 mixtures of both were fed at 0.1 ppm and 0.3 ppm for 30 days.

No OXY was detected in milk from the dairy cattle (sensitivity 0.005 ppm) after 30 days of feeding at these rates. OXY was occasionally detected in omental and back fat of dairy cattle at levels up to 0.02 ppm. The maximum ratio of metabolite level in fat to level of CHL fed was 0.07; 67% of the observations resulted in a ratio at or below 0.03. Analysis of brain and liver tissues from the same animals showed no detectable OXY.

In beef cattle, OXY was detected in omental and back fat at levels up to 0.03 ppm, representing a maximum Storage Concentration Ratio of 0.10. None of the metabolite was detected in liver or brain of these animals.

ABSENCE OF OXY IN PLANTS AND SOIL

Thus far we have observed no evidence of occurrence of OXY in plants or in soil from supervised field trials with Technical CHL, even where substantial other residues are expected and detected. Fig. 2 is a gas chromatogram (11% OV-17, QF-1 column) from residues on sugar beets. This is typical of observations on 22 samples of sugar beets, soybean stalks, flax straw and soil from either foliar or soil treatments at 1-12 lb. per acre.

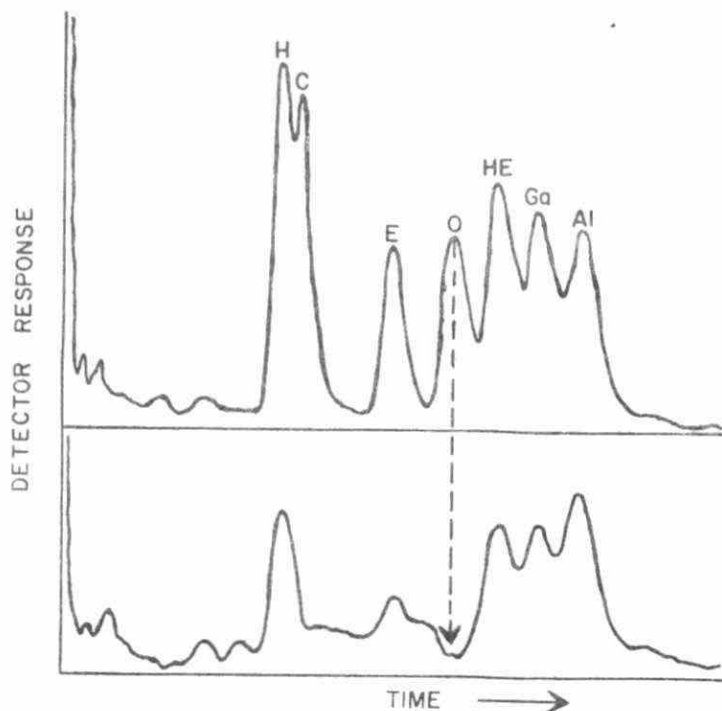


Fig. 2. Chromatograms: Upper. Standards: H, heptachlor; C, E, minor constituents of Tech. CHL; O, OXY; HE, heptachlor epoxide; Ga, γ -CHL; Al, α -CHL. Lower. Residue on sugar beets grown in soil treated with Tech. CHL, 12 lb/A.

SUMMARY AND DISCUSSION

Oxychlordanes, $C_{10}H_4Cl_8O$, (OXY) has been detected in and isolated from the fat of animals fed pure isomers of chlordanes (CHL). The propensity for storage is low. The ratio of storage level in fat to feeding level, an index of probability of amplification, is about 0.1 in a 30-day feeding of CHL and approaches roughly unity for chronic 2-year feeding of CHL. α -CHL appears to exhibit a somewhat smaller propensity of formation and storage of OXY than does γ -CHL.

OXY has not been detected in plants or soil treated with Technical CHL in supervised trials.

At feeding levels corresponding to proposed international tolerances, OXY is not detected in milk but may be found in the fat of cattle at or below 0.03 ppm.

ACKNOWLEDGMENT

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SOURCE: Environmental Quality - The Second Annual Report of the Council on Environmental Quality (August, 1971).

Table A-6

Dietary Intake of Selected Pesticides

Compound	FAO- WHO accept- able daily level mg./kg. ¹	1964-65			1965-66			1966-67			1967-68			1968-69			1969-70		
		Daily mg.	Intake mg./kg.	Per- cent ac- cept. in- take ²	Daily mg.	Intake mg./kg.	Per- cent ac- cept. in- take ²	Daily mg.	Intake mg./kg.	Per- cent ac- cept. in- take ²	Daily mg.	Intake mg./kg.	Per- cent ac- cept. in- take ²	Daily mg.	Intake mg./kg.	Per- cent ac- cept. in- take ²	Daily mg.	Intake mg./kg.	Per- cent ac- cept. in- take ²
Aldrin		.001			.007			.001			T			T			.1		
Dieldrin		.005			.057			.004			.004			.005			.005		
Total	.0001		.00009	90.0		.00013	110		.00006	60.0		.00006	60.0		.00007	70.0		.00007	70.0
Carbaryl	.02	.150	.0021	10.5	.026	.0005	2.50	.007	.0001	500			0	.003	.00004	.200			0
DDT		.031			.041			.026			.019			.016			.015		
DDE		.018			.028			.017			.015			.011			.010		
TDE		.013			.018			.013			.011			.005			.004		
Total	.01		.0009	9.00		.0010	10.0		.0008	8.00		.0007	7.00		.0005	5.00		.0004	4.00
Lindane	.0125	.004	.00007	.560	.004	.00006	4.80	.005	.00007	.560	.003	.00004	.320	.001	.00002	.160	.001	.00002	.160
Heptachlor		>.001						>.001			>.001			>.001			>.001		
Heptachlor epoxide		.002			.003			.001			.002			.002			.001		
Total	.0005		.000033	6.60		.00005	10.0		.000021	4.20		.000031	6.20		.000031	6.20		.000021	4.20
Malathion	.02			0	.009	.001	.500	.010	.0002	1.00	.003	.00004	.200	.012	.0002	1.00	.003	.0002	1.00

¹ mg./kg. = mg daily intake ÷ 69.1 kg. (average weight of 15 to 19-year-old male).

² Percent accept. intake = [mg./kg. actual ÷ mg./kg. FAO-WHO] × 100.

Source: Inner City Fund.

*

"ACCEPTABLE DAILY INTAKE

"The acceptable daily intake of a chemical is the daily intake which, during an entire lifetime, appears to be without appreciable risk on the basis of all the known facts at the time. It is expressed in milligrams of the chemical per kilogram of body weight (mg/kg).

"Explanatory note

"For this purpose 'without appreciable risk' is taken to mean the practical certainty that injury will not result even after a lifetime of exposure...."

Field Study of a Chlordane Residue Problem in Milk

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In January, 1968, State regulatory officials found unacceptable levels of what they described as heptachlor epoxide in the milk supply of the Gallatin Valley of Montana. In the following months, almost one-third of the dairymen in this milk shed were restrained from selling milk, approximately 1,500 head of cattle were quarantined and some cheese was seized and destroyed.

The Quest For Information

Immediately following the issuance of the first restraining order, there were innumerable requests from dairymen for information. Questions asked most frequently were: (1) how can the residue most quickly be eliminated from the milk, (2) where can hay and milk samples be analysed, (3) how reliable are the test results, (4) what level of residue can be fed, (5) what about the soil and the next hay crop, (6) what is the relationship of residue in milk fat to the level in body fat, and (7) what methods are available for determining when quarantined animals are acceptable for slaughter?

In addition, research personnel were puzzled about the level of heptachlor epoxide being reported in the milk when chlordane was the chemical used on the alfalfa fields.

The Recommendation To Use Chlordane

Chlordane had been recommended for use in the control of adult alfalfa weevil by the state entomologist. It was approved for such use by the U. S. Department of Agriculture from 1950 to January 8, 1968 (2), under the

Journal article No. 142 of the Montana State University Agricultural Experiment Station.

label which read in part, "make application in spring when the alfalfa is coming out of the ground and is about 1 to 1½ inches tall. Do not apply during blooming period. Do not feed treated forage to dairy animals or animals being finished for slaughter".

This had been interpreted by the state entomologist to mean application prior to the plant being 1½ inches tall did not constitute forage treatment (1). The Pesticide Regulation Division of the U. S. Department of Agriculture subsequently claimed this interpretation was incorrect (3).

Persistence Of The Residue In The Milk

Claborn had shown in 1953 (4) that chlordane (a) accumulated slower, (b) never reached as high a concentration and (c) was depleted faster from the body fat of animals than aldrin or dieldrin.

Table 1 shows the depletion rate of "apparent" heptachlor epoxide from the milk of some commercial herds in Montana, following the placing of these herds on feed that had been tested and found to be free of chlordane residue.

TABLE 1

Depletion Rate of (apparent) Heptachlor Epoxide from Milk (fat) Following Change to Uncontaminated Hay

Producer No.	1st Sample		2nd Sample		3rd Sample		Reduction In PPM	Days
	Date	PPM	Date	PPM	Date	PPM		
1	3-6	0.42	3-26	0.16			0.26	20
2	3-8	0.49	3-26	0.42	4-3	0.28	0.21	26
3	2-20	1.43	3-19	0.59	4-3	0.43	1.00	43
4	2-26	0.60	3-26	0.29			0.31	29
5	3-7	0.32	3-27	0.28			0.04	20
6	2-26	0.33	3-20	0.25			0.08	23
7	2-20	0.57	3-18	0.25			0.32	26

These data were taken from the report of the Montana regulatory agency (5) and show the results on the date samples were taken. More frequent sampling might have shortened the time required to reduce the residue to below the action level of 0.3 ppm in the milk fat.

However, most dairymen who followed the recommendation of feeding only those feeds known to be free of chlordane residue reduced the apparent heptachlor epoxide residue level of their milk (fat) to below the action level in 20 to 30 days. Where the initial level was relatively high (producer No. 3) a somewhat longer period was required.

Reliability Of Laboratory Results

Early in this situation the reliability of laboratory results, all of which were made by gas-liquid chromatographs without confirmation, became a matter of concern.

In an attempt to locate reliable analytical facilities available to individual dairymen, five samples were split and submitted to three different laboratories. One sample was submitted to a fourth laboratory. The results using gas-liquid chromatography without confirmation are shown in Table II.

TABLE II
Results Of Analysis Of Split Samples
By Different Laboratories

Sample No.	Product	PPM Chlordane				PPM (apparent) Heptachlor Epoxide			
		Laboratory				Laboratory			
		1	2	3	4	1	2	3	4
A	Milk (fat)	0.0	0.03	0.02		0.036	0.04	0.03	
B	Milk (fat)	0.153	0.12	<u>0.02</u>	2.54	0.129	<u>0.0</u>	0.16	0.59
C	Milk (fat)	0.0	0.02	0.02		0.0	0.0	0.01	
DD	Hay	<u>0.10</u>	0.0	0.01		0.0012	0.0	0.005	
E	Grass	<u>0.14</u>	0.0	0.01		0.004	0.0	0.005	

These results showed apparently good agreement on Sample A. On sample B laboratory No. 3 was low on the chlordane result (underlined) and laboratory No. 2 was low on the heptachlor epoxide result. Laboratory No. 4 was exceptionally high on both values. Agreement was good on sample C but on sample D and E, laboratory No. 1 was high on the chlordane values.

If the residue (ppm) for each of the first three laboratories is totaled and the variation between laboratories determined by the method of Snedecor (6), we find the mean result for chlordane to be 0.2143 ppm with a standard deviation of ± 0.4001 . This is a coefficient of variation of 186%. The mean value for heptachlor epoxide was 0.1400 ± 0.6828 , which is a coefficient of variation of 485%.

Stull *et al.* (7) found similarly high variability in working with DDT residues in milk from individual cows but suggested that pooled herd milk should be less variable. However, in a later report (8), where DDT intake was compared to levels in the milk of a herd of 500 cows, they found wide fluctuations and unexplainably high levels of residue in the milk.

Level of Chlordane Residue Advisable To Be Fed

No information was available relative to what level of chlordane residue might be fed and still keep the residue level in the milk below the action level. This was critically needed since a large amount of hay was being held in hopes it could be fed. We had been advised that possibly 0.02 ppm of residue in the hay might be maximum (9).

Thus, in September, 1968, twelve cows were placed on hay that had been analysed in March and reported to contain 0.25 ppm of chlordane. Feeding continued until December 1968. The hay was sampled by a core sampler, drilled approximately 18" to 20" into the end of each bale as it was fed. Samples from each bale were composited and analysed along with a milk sample, which was composited from the morning and evening milking of the twelve cows -- approximately every seven days for 16 weeks. A normal grain ration was fed, which on periodic sampling consistently gave negative results for chlordane residue. The results are shown in Table III.

These results show hay sprayed in April or May of 1967, harvested the early part of July and stored in an open stack, contained 0.25 ppm of chlordane residue in March of 1968, based on one sample. Starting in September of that same year and continuing for 16 weeks no sample ever reached 0.25 ppm, the highest level being 0.182. Six weekly samples were negative and the average residue (using 0.01 ppm for the negative values) over the 16 week period was 0.065 ppm. Milk produced from cows fed this hay resulted in no significant change in heptachlor epoxide residue, the average residue being 0.099 ppm on a fat basis. Analysis showed the coefficient

ent of variation for the hay samples to be 53.7% and for the milk samples 23.16%.

In-as-much as this level of residue in the hay did not produce any appreciable change in the residue in the milk a second group of six cows was started in September 1968 on hay that had tested 18 ppm of chlordane residue in February 1968. This group was handled in the same manner as the one above and continued on this feed for 12 weeks. The results are shown in Table IV.

TABLE III

Level Of Chlordane In Hay And (apparent)
Heptachlor Epoxide In Resulting Milk (fat)

Date	Hay Chlordane PPM (as fed)	Milk Heptachlor Epoxide PPM (fat)	
		Cattle on Treatment	Balance of Herd
March 1968	0.25*	-----	-----
8-8-68**	0.092*	-----	.054
8-15-68	Neg.(0.010)***	0.095	
8-22-68	0.122	0.090	
8-29-68	Neg.(0.010)	0.076	
9-5-68	0.177	0.067	
9-12-68	Neg.(0.010)	0.095	
9-20-68	Neg.(0.010)	0.079	.062
9-27-68	Neg.(0.010)	0.085	
10-3-68	Neg.(0.010)	P.Tr. 0.010	
10-15-68	0.085	0.056	
10-23-68	0.182	0.141	
10-29-68	0.011	0.149	
11-5-68	0.055	0.155	
11-13-68	0.105	0.097	.087
11-20-68	0.025	0.147	
11-26-68	0.022	0.131	
12-6-68	0.171	0.171	.056
AVERAGE	0.065	0.099	

* Stack samples

** Feeding contaminated hay began

*** Residue in hay fed previous period, i.e. 8-8-68 to 8-15-68

It will be noted that the level of chlordane in the hay varied over a wide range but never exceeded 11.9 ppm.

Yet, a portion of the original sample, stored in a closed metal container, still gave essentially that same (18 ppm) value in January 1969. Thus, as previously indicated, there appears to be a significant decrease in the chlordane level when hay is stored in an open stack.

TABLE IV

Level Of Chlordane In Hay And (apparent)
Heptachlor Epoxide In The Resulting Milk (fat)

Date	Hay Chlordane PPM (as fed)	Milk Heptachlor Epoxide PPM (fat)	
		Cattle on Treatment	Balance of Herd
9-68	18.0*		
10-9-68**	----	.095	.084
10-15-68	3.729***	1.338	
10-23-68	.476	.271	
10-29-68	1.806	.261	
11-5-68	2.973	.478	
11-13-68	1.405	.614	
11-20-68	.672	1.140	.064
11-26-68	.078	.208	
12-6-68	1.043	.154	
12-12-68	1.080	.340	
12-19-68	8.074	.180	
12-26-68	11.902	.763	.032
1-3-69	8.281	.876	
AVERAGE	3.549	.551	

* Stack sample

** Feeding contaminated hay began

*** Residue in hay fed the previous period, i.e. from
10-9-68 to 10-15-68

An analysis of these data show the mean value for the twelve samples of hay to be 3.459 ppm of chlordane, with a minimum level of .078 and a maximum of 11.902 ppm. The standard deviation was ± 2.4433 and the coefficient of variation 70.61%.

For the milk, five of the twelve samples were below the action level and seven above. The mean value was 0.551 with a minimum of 0.180 and a maximum of 1.338. The standard deviation was ± 0.2555 and the coefficient of variation 46.29%.

These two feeding trials would indicate chlordane residue in the hay in excess of 0.065 ppm may be tolerated without the apparent heptachlor epoxide exceeding the action level of 0.3 ppm but that chlordane residues in the hay averaging 3.45 ppm would result in milk exceeding the action level and thus be subject to removal from the market.

Summary and Discussion

This experience seems to point out that the continued registration and approval of the ambiguous language on the label for chlordane by the U. S. Department of Agriculture, Pesticide Regulation Division, particularly after terminating registrations for other organochlorine pesticides on alfalfa, was unfortunate. Our experience supports the work of Claborn of 1953 (4) in that chlordane did not build up to very high levels in the animals and was dissipated relatively quickly once the animals were taken off the contaminated feed. Also, we found a wide range in values reported by different laboratories analysing the same sample. Also, wide ranges in laboratory results when one laboratory follows the feed and milk residues on control feeding trials, as did Stull *et al.* (8).

Based on our results, as well as others (7,8), it is doubtful if the one sample procedure used by the regulatory agency as a basis for legal action on highly perishable products such as milk, where the product cannot be held until analytical results are available, is justifiable. A more realistic approach would seem to be the three out of five procedure used by the U. S. Public Health Service in evaluating other violations of milk production procedures, *i.e.* temperature, standard plate count and coliform count of fluid milk. Applying this procedure to the data in Table IV such milk would not have been removed from the market until November 13, 1968, when three of the last five samples were above the action level of 0.3 ppm in the milk fat. This would be more acceptable to the milk producer than a "single sample" procedure of removing the milk from the market on October 15, 1968, permitting it to again be sold from October 23 to November 5 and then taking it off again on November 5. Following November 5 this milk source, using the three out of five approach, would not again be permitted to be sold for the duration of the period, even though on November 26, December 6 and December 19 the analysis are below the action tolerance. This would provide considerably more assurance that the consumer is getting a safe milk supply.

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*Evaluation and Bibliography—by
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Evaluation

The critical developments with respect to technical chlordane have not been readily apparent to the general scientific community. The reason for this may be that many developments in the history of this product have been recorded in publications other than the formal scientific literature. Scientific observations reported in publications such as the United States Federal Register or in United States Department of Agriculture Research Service Reports are generally obscured from the broad scientific community. Recent entries in the literature reflect this lack of awareness about the nature of technical chlordane and its residues.

Evidence on agricultural use of technical chlordane has recently been subjected to intense scientific examination. An Advisory Committee of five eminent scientists was appointed by the United States Food and Drug Administration to review a proposal to repeal tolerances for residues of chlordane. The Committee was appointed by the FDA from a panel nominated by the National Academy of Sciences-National Research Council. After considering information presented by various parties, the Advisory Committee issued a report in February, 1965. On the basis of that report the Food and Drug Administration published a

final order in the Federal Register in April, 1965, retaining the tolerances of chlordane. The subject matter considered by the Advisory Committee on chlordane tolerances is germane to the problem before this Commission, namely, the terminal residues of chlordane. I have therefore appended copies of the report of the Advisory Committee on tolerances of chlordane, and also a compilation of excerpts from chemical evidence presented to the Committee. (The compilation of evidence here carries the title "Chlordane: Composition, Analytical Considerations, and Terminal Residues".)

It is apparent that to assess the problem of the chemical nature of terminal residues of chlordane in a general way, one must start with consideration of constancy of the product applied, and then proceed to evaluate formulations and to appraise the contributions of regional climates and other agricultural and agronomic factors. The information assembled for the Chlordane Advisory Committee involves evaluation of factors that influence terminal residues. The Committee concluded that technical chlordane, since 1950, is a uniform product capable of toxicological and agricultural evaluation. The Committee states, in part, "The Committee has no reason to assume that the composition of chlordane is less rigidly controlled than the composition of other mixtures that are successfully used as agricultural chemicals or indeed allegedly pure compounds that are likewise successfully employed for a variety of purposes."

Analytical methods are required for independent verification of the integrity of technical chlordane and its formulations. I have submitted as part of my bibliography a number of methods which encompass such techniques as chemical determination of total organic chlorine, colorimetric reactions, spectrophotometry in the ultraviolet, visible, and infrared regions, and gas chromatography. Several of these methods have become official methods after collaborative study by the Association of Official Analytical Chemists in the United States and the European Commission for Methods of Analysis of Pesticides (CEMAP). The committee states, with regard to residues, "In

the case of technical chlordane, despite the difficulties inherent in a mixture, the current analytical needs for residue determinations appear to have been met on the basis of various determinations: total organic chloride above control, response in colorimetric reactions, and gas chromatographic determinations of residues, including the quantitation of the more persistent components as so-called 'signature peaks'. Electron capture gas chromatography is used in analysis of chlordane residues. Sequences of analyses on samples taken after the agricultural application of technical chlordane exhibit a consistent pattern. Initial residues correspond very closely to technical chlordane, as would be expected. Subsequent chromatograms indicate a loss of more volatile components so that eventually two peaks dominate the chromatogram. These are the "signature peaks" and correspond to the retention time of known isomers of chlordane. Neither heptachlor, a constituent of technical chlordane, nor heptachlor epoxide is apparent in the chromatogram of the terminal residue.

Fundamental knowledge regarding terminal residues of chlordane is now available. The evidence appears to reveal principles which are applicable in world-wide use of chlordane. It may be necessary, however, to quantitatively measure the regional effects of climate and other agricultural factors by appropriate experiment. Except for such regional measurements, however, it appears that the chemical nature of the residue is sufficiently known to simplify for FAO and WHO their assessment of toxicological data for the establishment of numerical values for acceptable daily intake and permissible levels.

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Evaluation—by P. B. Polen (Velsicol Chemical Corp., 330 E. Grand Ave., Chicago, Ill. 60610, U.S.A.)

A study of the aging of chlordane residues has recently been reported by Klein and Link (1), who demonstrate the progressive simplification of the residue pattern with time and the eventual dominance of two peaks which, while not named, appear to correspond to α - and γ -chlordane. Fragmentary information indicates that food processing is a probable means of removing residual chlordane from food. Experiments at the scales of pilot plant and commercial production of edible vegetable oils have shown that chlordane residues present in the crude vegetable oil are completely removed. Process stages designated as deodorization and hydrogenation appeared to be particularly effective in producing the residue-free food products (2). Rapid degradation of chlordane in soil has been observed by Jones and is attributed to microbial effects (3). Chemical analysis gives higher apparent residue values than bioassay of the same soil; this discrepancy is attributed to the biological inactivation of chlordane by the soil (4).

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EXHIBIT VI (C)

Evaluation—by P. B. Polen (Velsicol Chemical Corp., 330 E. Grand Ave., Chicago Ill. 60610, U.S.A.)

In a search for the quantitative regional influences and the effects of cooking upon chlordane residues, a study has been completed for foliar applications to beans and cabbage in seven locations within Europe and North America. The results indicate that climatic conditions do not significantly influence the composition, pattern of dissipation, or levels of residues when compared with other experimental variables (1). Simple cooking of the beans or cabbage by boiling for 10 minutes in salted water also had no significant effect on levels or composition of the residues. For milk processing operations as in production of condensed milk, dried milk, or evaporated milk reduce the residues by 25 to 50% (2). The earlier work of Gooding (3) has been supported by further evidence that the processing of edible vegetable oils under commercial conditions removes residues of several pesticides, including chlordane (4). Studies are in progress to determine the identity of the separate metabolites which arise from alpha- and gamma-chlordane isomers; these have gas chromatographic characteristics similar to those of heptachlor epoxide but not identical.

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3. Terminal Chlordane Residues

The Commission reviewed work in progress on the metabolism of chlordane in animals, on the toxicology of α - and γ -chlordane, and on hydrophilic metabolites in plants. The Commission noted that the terms α - and γ -chlordane have now been changed to *cis* and *trans*. Arrangements were made to continue collation of information on the terminal residues of chlordane.

Evaluation—by P. B. Polen (Velsicol Chemical Corp., 330 E. Grand Ave., Chicago, Ill. 60611)

A number of projects are in progress, but are at present incomplete, with respect to terminal residues of chlordane. Manuscripts are in preparation on: (a) Terminal Residues of Chlordane (I): Relationship to Technical Chlordane (1); (b) Terminal Residues of Chlordane (II): Weathering of Residues from Foliar Treatments with Technical Chlordane (2); (c) Terminal Residues of Chlordane (III): Characterization of Components of Technical Chlordane (3). Chemical research is being conducted on the isolation, identification, and properties of unknown metabolite(s) of chlordane in animals (3) and a series of pharmacological investigations on α - and γ -chlordane are also in progress (4) to supplement chemical knowledge on residues.

Bevenue and Yeo (5) have reported on changes in composition of technical chlordane dispersed in water or when vapors are exposed to water. The evolution of chromatographic patterns is similar to observations reported to this Commission and the authors suggest that the chlordane peaks, which we have called "signature peaks," be used to quantitate residues. The authors ap-

pear to have been unaware of published IUPAC Commission reports (1, 2). Saha and Lee (6) report mass and infrared spectroscopic data on isolates from a commercial chlordane formulation (25% granules). Their interpretations confirm the identity of major components, but their identification of some minor components and their assignment of structures differ from the conclusions of other workers (3, 7). Chau and Cochrane (8) have described specific chemical methods for gas chromatographic identification of several organochlorine pesticides, including constituents of technical chlordane. Additional identifications, effects of strong basic reagents, and structures of chlordanes (and others) are discussed by Cochrane (7).

To provide a reference for ascertaining continuing constancy of composition of technical chlordane, a brochure (9) has been compiled containing physical, chemical, and biological test methods, a statement of approximate composition, and specifications.

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2. Terminal Chlordane Residues

The Commission received a report on work completed on the terminal residues of chlordane. An animal metabolite of chlordane, called oxy-chlordane ($C_{10}H_4OCl_8$), has been isolated from the fat of pigs fed massive doses of the pure isomers and from milk and cheese produced from cows feeding on alfalfa contaminated with chlordane. Thus far all evidence indicates that oxy-chlordane is formed only in animals. It was noted that chlordane residues found in the cuticle of root crops such as potatoes, even after washing, can be accounted for by the incorporation of minute particles of soil in the skin waxes. The Commission noted that chlordane will be reviewed at the 1970 FAO/WHO Joint Meeting, after which an indication should be forthcoming as to whether their requirements on chlordane have been met.

Evaluation—by P. B. Polen (Velsicol Chemical Corp., 341 E. Ohio St., Chicago, Ill. 60611)

(A) Animal Metabolite of Chlordane

Analytical evidence for the existence of unrecognized metabolites from pure isomers of chlordane was reported to this Commission at its 3rd meeting, Sittingbourne, Kent, England, Oct. 1968 (1). At that time different metabolites from each of the isomers were suspected. Since then an animal metabolite ($C_{10}H_4OCl_8$) of chlordane, hereafter called oxychlordane (OXY), has been isolated from the fat of pigs fed massive doses of the pure isomers (2) and from milk and cheese produced from cows feeding on alfalfa contaminated with chlordane (3). OXY has been synthesized and a structure has been proposed. Either α - or γ -chlordane yields the same metabolite, identical to the synthetic product when judged by analytical parameters (IR, NMR, GLC, TLC), but there are indications that enantiomorphic selection occurs during metabolism (2, 4).

Analytical characteristics of OXY have been described and its propensity for storage in the fat of animals has been studied (4). The storage concentration ratio (residue level in fat divided by level in feed) is a maximum of 0.1 in back fat of cattle fed chlordane for 30 days, 0.24 in pigs fed 90 days, and approximately unity in rats or dogs fed chlordane 1 to 2 years. OXY was not detected in milk of dairy cattle fed up to 0.3 ppm chlordane (maximum FAO/WHO tolerance)

for 30 days. From the relatively low storage concentration ratios observed in supervised feeding trials, it was concluded that the probability is low for amplification of residues in the food chain via a chlordane/oxychlordane mechanism.

OXY was not detected in residues in soil and plants; evidence indicates thus far that OXY is formed only in animals and does not occur as a constituent of terminal chlordane residues on crops.

(B) Nomenclature of Chlordane Isomers

Three systems are now used in the literature to name the principal isomers of chlordane (whose gas chromatographic peaks have been called "signature peaks" (5)). The oldest system has been used since about 1947 by Velsicol, in whose laboratories chlordane was developed. This nomenclature (hereafter System 1) was employed in preparing technical literature, in documents for regulatory agencies, and in identification of reference analytical standards which were distributed to university researchers, regulatory laboratories, and analysts. System 2 appeared in the technical literature in the early 1950's; System 3 was first used in 1969.

Correct recognition of this nomenclature is essential also to interpretation of literature of technical heptachlor, since γ -chlordane is sometimes a component of its terminal residues (6).

The systems are correlated below and some literature utilizing each is cited:

System 1 (7, 8, 9, 10)	System 2 (5, 11, 12, 13)	System 3 (14)
α -chlordane	β -chlordane	<i>cis</i> -chlordane
γ -chlordane	α -chlordane	<i>trans</i> -chlordane
	γ -chlordane*	

* Reference 12 only. This is *not* one of the "signature peaks" isomers.

Unequivocal bases for experimental distinction of the 2 isomers are the gas chromatographic retention times (α -chlordane has the longer retention time) and IR spectra.

(C) Photoreactions in Terminal Residues of Chlordane

The possibility of conversion of constituents of residues from technical chlordane by UV *in vitro* has been demonstrated by Korte (15), Benson (17), and Rosen (18) and their co-workers.

Whether or not these transformations are of significance in altering the composition of terminal chlordane residues under agricultural conditions needs further evaluation.

Observations of the IUPAC Chlordane Working Party on gas-liquid chromatograms from foliar applications to beans and cabbage appear to give no new peaks during the aging of the residues (1) and hence gave no indication of photolytic products. A more recent investigation directed specifically toward evaluation of the effects, if any, of sunlight on chlordane residues also finds no significant indications of the production of photolytic products under typical field conditions (19).

(D) Effects of Food Processing on Chlordane Residues

Saha and others (6, 20, 21) have consistently shown that chlordane residues from soil treatments of root crops with either technical chlordane or technical heptachlor, in which γ -chlordane is a component, are concentrated in the peel and that peeling and/or cooking reduces or eliminates the residue.

Bevenue and Yeo (22) have demonstrated the effects of processing on removal of chlordane residues from grain products. Wheat flour and rice, each contaminated by exposure to chlordane vapors, were processed to produce 2 cooked products: a baked cookie and boiled rice. The baked wheat flour cookie contained an average of 50% less residue than the wheat and the cooked rice about 73% less than the raw product.

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Evaluation—by F. Korte (Institut für Ökologische Chemie der Gesellschaft für Strahlenforschung mbH München, D 5201 Schloss Birlinghoven, Germany)

Metabolism of trans-Chlordane in White Cabbage and Carrots

White cabbage leaves quickly take up *trans*-chlordane after foliar application. Four weeks after application less than 1% of the residues was found on the leaf surfaces, more than 90% of the residues was found in the leaves. Ten weeks after application total residues amounted to merely 20% based on the applied radioactivity. This means that residues disappear much slower than in the case of the related substance heptachlor.

Soil treatment (carrots) with *trans*-chlordane only resulted in low residues in carrots, namely 0.01 ppm in carrots themselves and 0.06 ppm in their leaves (12 weeks after application). Residues in carrots were mainly metabolites, whereas residues in leaves (about 2/3 of the radioactivity) consisted of *trans*-chlordane.

Although these tests, carried out under greenhouse conditions which were not under strict control, cannot be compared quantitatively with tests carried out under practical conditions, the results reveal nevertheless that a considerable part of the residues is present in the form of conversion products after chlordane application.

3. Terminal Cyclodiene Compound Residues

The Commission reviewed progress on the knowledge of the nature of terminal residues and photochemical reaction products of cyclodiene insecticides. An important development was the confirmation of the bridged ketone structure of Matsumura's dieldrin metabolite F by means of its synthesis. It was noted that the number of metabolites of cyclodiene compounds is increasing and that the need exists to synthesize these compounds in order to evaluate their importance as terminal residues under field conditions. Photo-dieldrin and photo-endrin are significant residues under certain conditions and should be included in any discussion of residues. The quantitative occurrence of hydrophilic metabolites and photo-transformation products under practical conditions needs to be reported. Arrangements were made to continue to collate and report results on the terminal residues of cyclodiene compounds.

Evaluation—by F. Korte (Institut für Ökologische Chemie der Gesellschaft für Strahlenforschung mbH München, D 5201 Schloss Birlinghoven, Germany) and P. E. Porter (Shell Development Co., P.O. Box 4248, Modesto, Calif. 95352)

An excellent review of the metabolism of cyclodiene insecticides, including literature through June 1968, has been prepared by G. T. Brooks (1). Some recent developments in relation to cyclodiene insecticide terminal residues are summarized in the following sections.

(A) Transformation in the Soil

Miles, Tu, and Harris (2) have continued their study of the metabolism of heptachlor and its degradation products by soil microorganisms. Heptachlor is epoxidized to heptachlor epoxide. It is also hydrolyzed to 1-hydroxy-chlordane which is epoxidized to 1-hydroxy-2,3-epoxy-chlordane. They now have evidence that the latter compound is oxidized to 1-keto-2,3-epoxy-chlordane. Lichtenstein *et al.* (3) examined soils which

had received aldrin or heptachlor either in one treatment at 25 lb/acre or in 5 annual treatments of 5 lb/acre per treatment, 10 years after the first treatment. In the case of the single aldrin treatment, the soil residues of aldrin plus dieldrin declined roughly logarithmically with time with approximately half of the residue disappearing each 2.25 years. In the heptachlor-treated soils, conversion to the epoxide was slower, but the sum of heptachlor and its epoxide declined logarithmically with time. The time for half disappearance was about 2 years.

Saha and Lee (4) treated fertile clay loam soil with ^{14}C -dieldrin. After one year, in which wheat plants and carrots were grown in the soil, they examined both plants and soil. It was evident that in 1 year in their soil, insignificant dieldrin conversion to other products had occurred. An important development in relation to the soil transformation of aldrin and dieldrin was the synthesis by Bienieck and Korte (5) of the bridged ketone structure which Matsumura *et al.* (6) proposed as their metabolite F. Matsumura *et al.* (7) incubated ^{14}C -labeled endrin with 150 cultures of microorganisms isolated from soils. Twenty-five appeared to be active in degrading endrin.

(B) Transformation in or on Plants

A number of pertinent studies have been made at the Institut für Ökologische Chemie, Schloss-Birlinghoven. Six weeks after the ^{14}C -endrin treatment, 32–45% of residues based on the applied radioactivity was found on and in the tobacco plants. The residues consisted not only of endrin but also of a very hydrophilic substance (30% after 6 weeks).

It was shown by radio-thin layer chromatography that besides endrin there were 2 groups of degradation products in cotton plants, one group being only slightly more hydrophilic than endrin itself, the other one very hydrophilic. After foliar application to white cabbage, residues of Δ -keto endrin disappear more slowly than those of endrin itself.

Ten weeks after foliar application of ^{14}C -isodrin to white cabbage, total residues amount to $\frac{1}{2}$ of the applied quantity. The main product in the plants was endrin; the main product on the leaf surfaces was a very hydrophilic substance which so far had been unknown. Leaves and leaf

surfaces contained about 20–30% of Δ -keto dieldrin.

In contrast to the tests with white cabbage, 12 weeks after soil treatment as much as 40% of the residues in soil was isodrin; carrots and carrot leaves also still contained isodrin after this period.

After foliar application of ^{14}C -photo-dieldrin to white cabbage, residues disappeared more slowly than in corresponding tests with aldrin, dieldrin, isodrin, and endrin. Metabolite fractions consisted of a very hydrophilic main product and at least 2 less hydrophilic by-products.

(C) Animal Metabolism

Observations on the animal metabolism of the cyclodiene insecticides suggest that microsomal enzyme systems in the liver must be responsible for their conversion to more hydrophilic metabolites. *In vitro* transformations by microsomal enzymes have now been amply demonstrated. Matthews and Matsumura (8) found that dieldrin is converted by rat liver microsomes *in vitro* into a number of more hydrophilic materials. The principal fecal dieldrin metabolite of rats and the principal rat urine metabolite were both major *in vitro* liver enzyme metabolites. The main *in vitro* metabolite of dieldrin was not identified.

Brooks *et al.* (9, 10) have demonstrated epoxide hydrolase activity in rabbit and pig livers even for the refractory dieldrin. Richardson *et al.* (11) fed rats with ^{14}C -labeled endrin and confirmed the rapid excretion pattern reported by Klein *et al.* (12). Baldwin *et al.* (13) have now found that the major fecal metabolite is a secondary alcohol formed by substituting a hydroxyl group for one of the hydrogens of the methano-bridge of endrin. Rat liver perfusion studies with endrin (14) confirmed that it is metabolized by enzymes of the liver. Dr. Korte's laboratories have found that mammals metabolize Δ -keto endrin as quickly as endrin itself. Hedde *et al.* (15) administered ^{14}C -dieldrin to sheep and about $\frac{1}{3}$ was excreted over 6 days with a little less than half of the excreted materials in the urine. Feces were not examined. The urine contained 6 metabolites, 4 extractable with hexane. Feil *et al.* (16) identified the urine metabolite as the *trans*-6,7-dihydroxy- Δ -hydro aldrin, and one as a monohydroxy dieldrin in which one methano-bridge hydrogen was replaced with a hydroxyl. Baldwin *et al.* (17)

isolated a larger and purer sample of the principal dieldrin feces metabolite of rats, which allowed a more accurate structure determination. This proved to be the same as the monohydroxy compound described by Feil *et al.*, with the methano-bridge of dieldrin hydroxylated. It is undoubtedly identical to the major fecal metabolite isolated by Matsumura. Baldwin and Robinson (18) fed the photo-isomer of dieldrin to rats for 13 weeks. They found that a metabolite was stored in the tissues in small amounts. This metabolite was identified as the ketone which is the principal rat urine metabolite of dieldrin. Dailey *et al.* (19) and Klein *et al.* (20) administered ^{14}C -photo-dieldrin to male and female rats intraperitoneally and orally daily, 5 days a week, for 12 weeks. Analysis of the urine showed the principal metabolite (about 85% of the total) of male rat urine to be the ketone commonly called "Klein's metabolite" in agreement with Baldwin and Robinson; however, none of this ketone was detected in female rat urine. No photo-dieldrin was excreted in the urine of either. At least 4 metabolites were present in the urine of female rats, but these have not been identified so far.

The following observations have been made in Dr. Korte's laboratories:

After intravenous application of ^{14}C -photo-dieldrin to rats (70 $\mu\text{g}/\text{kg}$ body weight), the radioactivity was excreted only slowly (about 17% by male rats within 72 hr, about 15% by female rats within the same time). Ninety-five per cent of the radioactivity found in feces and urine was due to 2 metabolites in the ratio 2:1. The main product is very hydrophilic; the second metabolite is only slightly more hydrophilic than photo-dieldrin itself. After intravenous application of ^{14}C -photo-dieldrin to rabbits (115 $\mu\text{g}/\text{kg}$ body weight), excreta contained about 16% of the radioactivity only in the form of a very hydrophilic metabolite after 96 hr. Korte and co-workers have also studied the metabolism of dihydroxy-dihydro aldrin. After intravenous or oral application, respectively, of this ^{14}C -labeled metabolite to rabbits, only slight conversion was found. In rats, however, there was a higher conversion rate of this substance and about 10% of the radioactivity excreted in the feces is due to hexachloro-tetrahydroindane-1,3-dicarboxylic acid. This result is the first example for the actual degradation of a compound of this class in mammals.

On feeding ^{14}C -heptachlor to rats and rabbits, Kaul *et al.* (21) found only heptachlor epoxide in the tissues. Twenty per cent of the radioactivity in the urine and feces was a hydrophilic metabolite which was chromatographically identical to the 1-hydroxy-heptachlor epoxide. Similar results were obtained for intravenous application of heptachlor epoxide. After injection of 1-hydroxy-heptachlor epoxide in male rats, no further change occurred.

Lawrence *et al.* (22) identified a metabolite in milk and cheese which resulted from feeding technical chlordane to cows. The compound was an octachlor epoxide.

(D) Photochemical Transformations

Photochemical transformations of the cyclodienes are important with respect to terminal residues on plants. McGuire *et al.* (23) studied the photochemistry of heptachlor under various conditions. Korte *et al.* (24, 25) reported photochemical transformations of many cyclodienes with and without sensitization by carbonyl compounds. Under conditions favorable to triplet formation, good yields of bridged or "caged" structures were obtained where such structures are possible. An important experiment isomerizing *exo-endo*-tetracyclo-(6.2.1 13,6 . 0 2,7)-dodecene-4 in hexadeutero-acetone proved that the rearrangement to form bridged molecules does not occur through a nonclassical homo-allyl radical as a transition state. Benson *et al.* (26) irradiated acetone solutions of chlordane and several of its pure components. In those cases in which a double bond is present in the 1,2-position, the cage structures were obtained. At this time information does not seem to be available relative to the significances of the various photochemical products as terminal residues.

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EXHIBIT VII

AGP:1970/M/12/1

1970 EVALUATIONS OF SOME PESTICIDE RESIDUES IN FOOD

THE MONOGRAPHS

Issued jointly by FAO and WHO

The content of this document is the result of the deliberations of the Joint Meeting of the FAO Working Party of Experts and the WHO Expert Group on Pesticide Residues, which met in Rome, 9-16 November, 1970.



FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
WORLD HEALTH ORGANIZATION
Rome, 1971

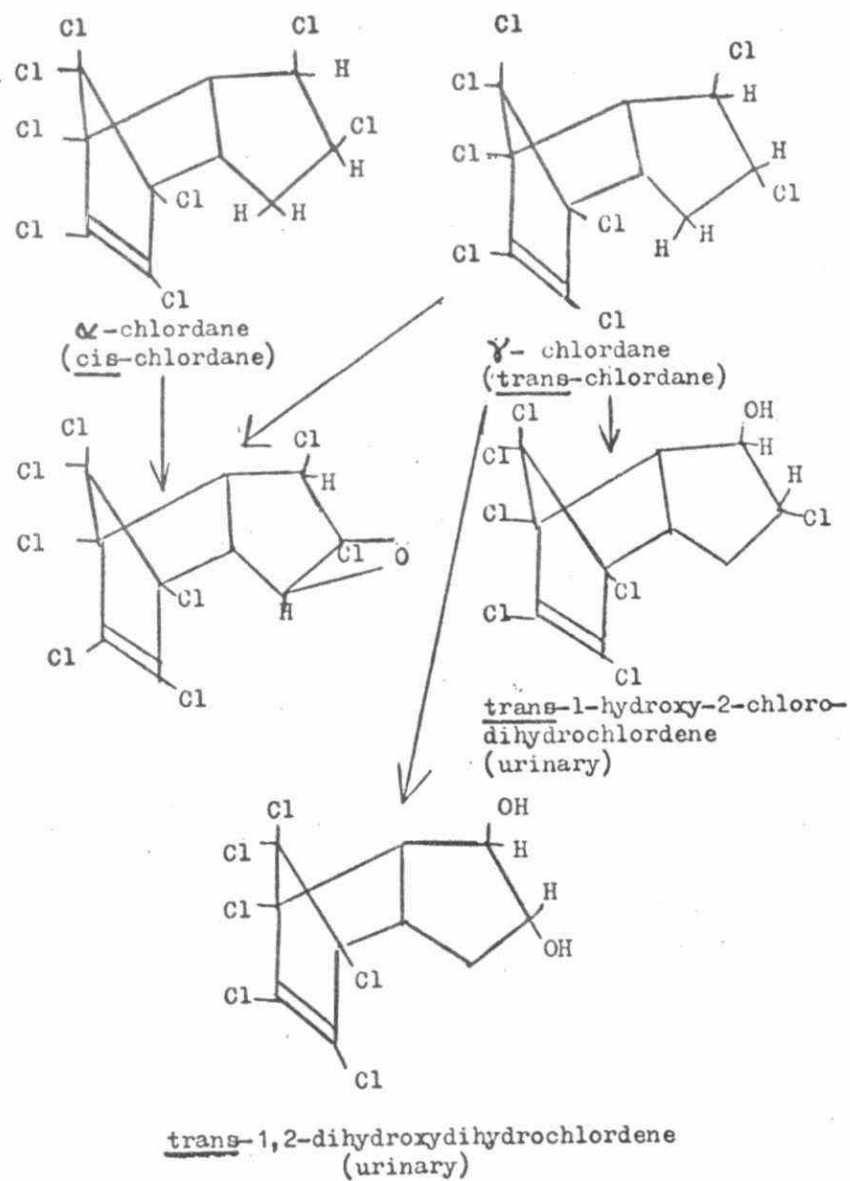


Figure 1.
Metabolism of chlordane in mammals.

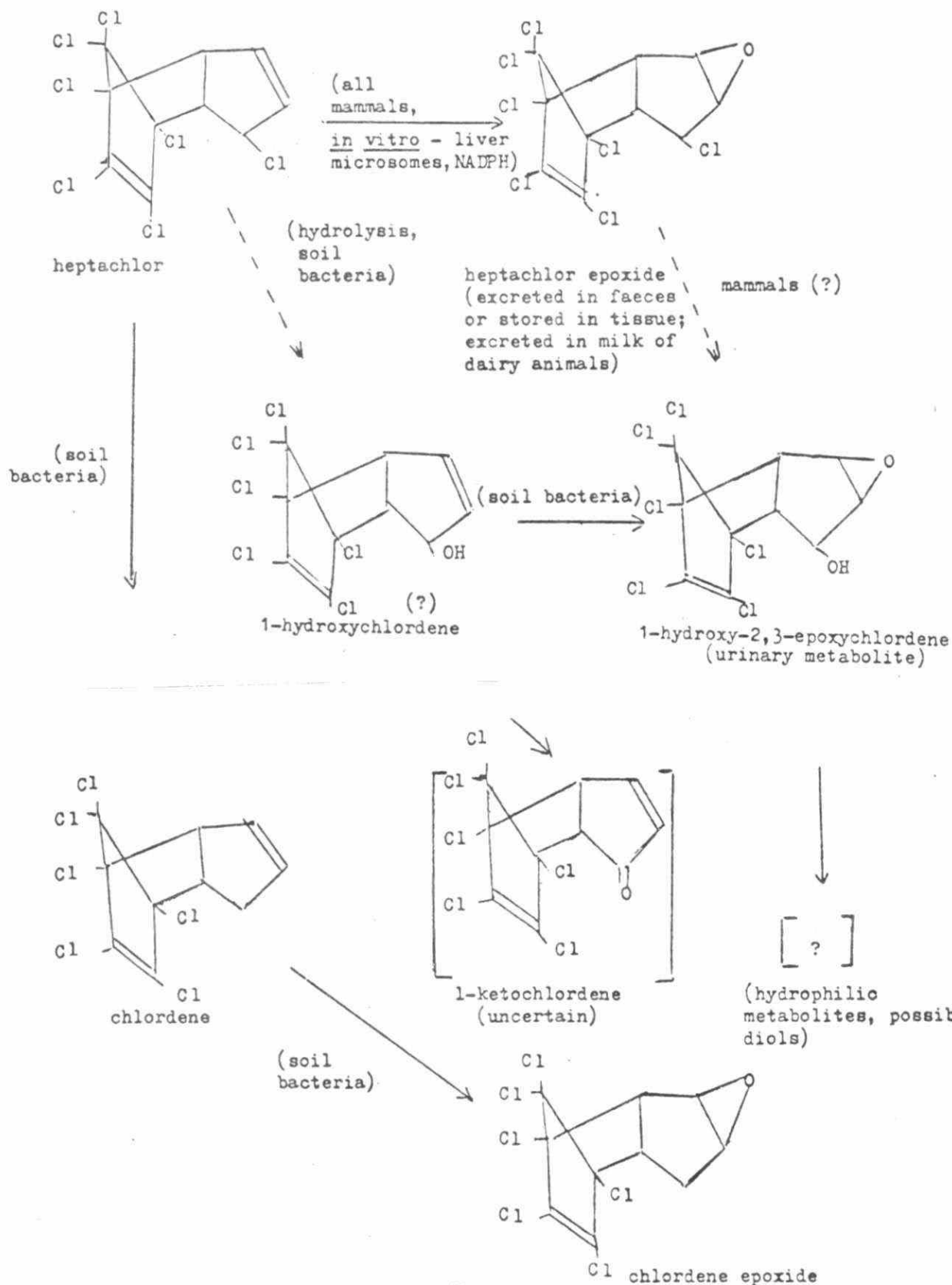


Figure 1
Metabolism of heptachlor

BEHAVIOUR AND PERSISTENCE OF HEPTACHLOR, TECHNICAL CHLORDANE, AND AG-CHLORDANE (HCS-3260) IN SOIL

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Research Institute
Canada Department of Agriculture
London, Ontario

The discussion today really covers three insecticides, Heptachlor, technical chlordane, and an experimental compound known as AG-Chlordane or by the code number, HCS-3260. Heptachlor was used for many years in Ontario for soil insect control and was one of the most effective soil insecticides available. The use of this compound was banned in 1969 in conjunction with bans placed upon other cyclodiene insecticides, such as aldrin and dieldrin. Since that time the use of technical chlordane, also a cyclodiene insecticide which was not banned, has increased markedly. Technical chlordane is commonly used for control of the northern corn rootworm, white grubs and wireworms and is available to both the agricultural and home-owner market. The third compound, AG-Chlordane is an experimental material which may be introduced as a replacement for the present technical chlordane. None of the compounds mentioned is available commercially as a "pure" material (Table 1).

Table 1. Insecticides involved in Heptachlor-Chlordane review

Heptachlor	- γ -chlordane, (heptachlor epoxide)
T. chlordane	- α -chlordane, γ -chlordane, nonachlor, heptachlor, (heptachlor epoxide)
AG-chlordane	- α -chlordane, γ -chlordane, heptachlor, (heptachlor epoxide)

Heptachlor contains about 20% γ -chlordane and this is usually the residue which is found in soil long after heptachlor and heptachlor epoxide have disappeared. In addition, heptachlor epoxide is also a break-down product of heptachlor and it is both insecticidal and persistent in soil. So when we use heptachlor we are basically concerned with these three residues in soil. Technical chlordane is a mixture of a considerable number of compounds - on a gas chromatograph chart you get about 20 peaks. Basically there are four compounds that are insecticidal, α -chlordane, γ -chlordane, nonachlor, and heptachlor and, again, because of the degradation of heptachlor you must be concerned with heptachlor epoxide. In AG-chlordane we are concerned primarily with α - and γ -chlordane and there is usually less than 1% heptachlor in the mixture. Conceivably, this very small amount of heptachlor in AG-chlordane could also lead to minute residues of heptachlor epoxide in soil.

The work which I will discuss today has been done in our own laboratory and is concerned primarily with the behaviour of the insecticides in soil or their persistence of biological activity. However, I have also included some residue data. Although we have a lot of data on a variety of pests of economic importance, I have tried to keep the discussion consistent by dealing only with one insect throughout the talk so that I can show parallels or differences which may occur among the various materials. In addition, to keep it simple, I have resorted to using the results of screening tests where we use a very broad range of concentrations, rather than detailing LD₅₀ values. The first insecticide which I will discuss is heptachlor (Table 2). As you can see from the data in Table 2, when heptachlor is compared with aldrin and DDT as a contact insecticide, all three are equitoxic.

Table 2. Relative Toxicity of Heptachlor to 1st Stage Crickets by Direct Contact and as a Soil Treatment

Insecticide	Av. corr. % mortality at indicated			
	% insecticide solution			
	0.001	0.01	0.1	1.0
heptachlor	0	0	100	100
aldrin	0	0	100	100
DDT	0	0	100	100

	ppm in moist mineral soil			
	0.1	0.5	1	5
heptachlor	40	100	100	100
aldrin	30	100	100	100
DDT	0	0	0	100

However, when you incorporate the insecticide into the soil, you get a remarkable difference in toxicity. DDT is not actually a very effective soil insecticide and, in these particular tests, it was necessary to go to a concentration of 5 parts per million in soil to obtain any effect. By contrast both heptachlor and aldrin were remarkably effective insecticides in soil. Of the two, we generally find that heptachlor is just a shade more effective than aldrin as is shown in the table. Although it does not appear that there is a great deal of difference in toxicity between the two materials, studies which we have done over the years, both in the laboratory and in the field, have always indicated that heptachlor is just slightly superior to aldrin.

Insecticidal activity in soil is dependent not only on the toxicity of the insecticide but on several soil and climatic factors (Table 3). The results shown in Table 3 indicate the importance of such factors as soil type and moisture. One of the major factors that influences the activity of an insecticide in soil is the soil type. In Table 3 I have listed some LD₅₀'s where I have taken two extremes, sandy loam and muck soil, which in one case contains about 1/2% organic matter and in the other case 65% organic

Table 3. Influence of Soil Type and Moisture on the Biological Activity of Heptachlor ¹⁾

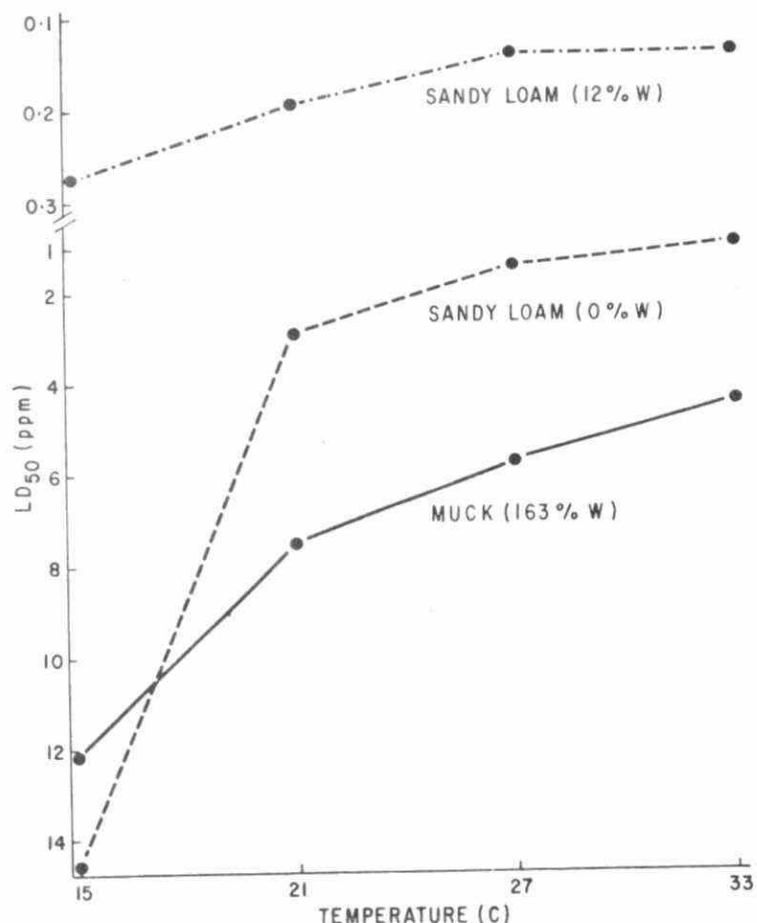
Soil type	% organic matter	LD ₅₀ (ppm)		Ratio: Dry/Moist
		Moist Soil	Dry Soil	
B. fine sandy loam	1.4	0.09	1.15	x 12.8
Muck	64.6	4.19	5.39	x 1.3
Ratio: Muck/Sand x 46.3			x 4.6	

1) Soil type and moisture influence the biological activity of heptachlor epoxide, T. chlordane, and AG-chlordane in a similar fashion.

matter. As you can see, heptachlor was extremely toxic in the moist mineral soil. It was necessary to go to a much higher dosage in muck to get an equivalent degree of activity. The heptachlor was approximately 46 times as effective in the sandy loam as in the muck. Another factor which will influence insecticidal activity in soil is the amount of soil moisture. The drier the soil, the less effective the material (Table 3). You will notice that in mineral soil, it was necessary to go from 0.09 ppm in moist mineral soil to 1.15 ppm in oven-dry mineral soil to obtain a LD₅₀ for heptachlor. The heptachlor was 12.8 x as effective in the moist soil as compared to the dry soil. In organic soils, the moisture effect is not as obvious and as shown in the data on Table 3, there was very little difference in the toxicity of heptachlor in oven-dry soil as compared to the moist organic soil. Moisture has less effect on the toxicity of heptachlor as compared to other materials which we have tested. Some of the new compounds, such as Furadan or diazinon, are one 1/100 as toxic in dry mineral soil as in moist soil. Similarly, soil type appears to influence the toxicity of some of the newer insecticides more strongly than with the cyclodiene insecticides. For example, Furadan will work very effectively in mineral soil but is so strongly inactivated in organic soils that, in some cases, it is very little value. Soil moisture and soil type are two factors which influence the biological activity of an insecticide in soil to a very marked extent. These factors also influence the biological activity of heptachlor epoxide, technical chlordane, and AG-chlordane in a very similar fashion.

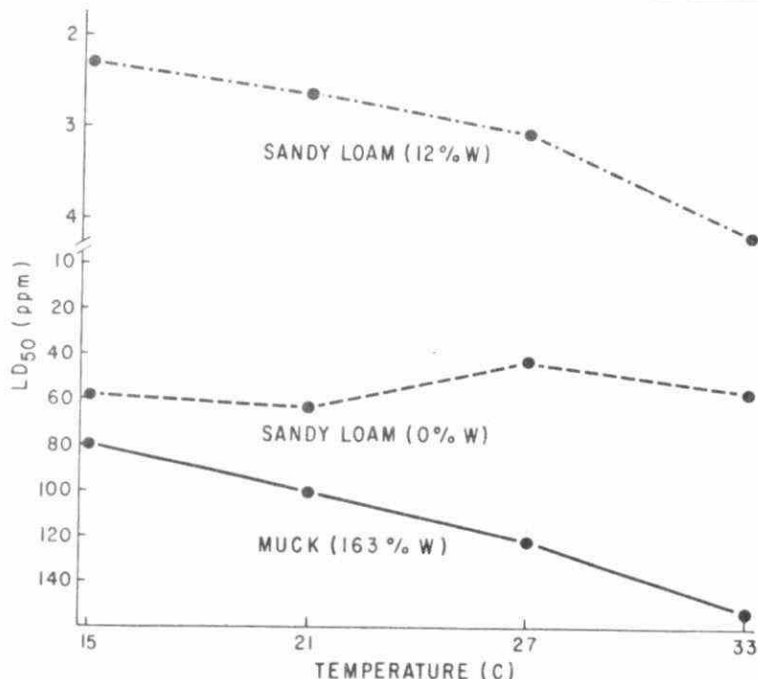
Another factor which will influence insecticidal toxicity in soil is soil temperature (Fig. 1). In the case of heptachlor, the insecticide becomes more toxic in all soils with increasing temperature. In other words, you have a positive temperature coefficient. As you can see from the data shown in Fig. 1, there is a considerable difference in toxicity of heptachlor between 15 and 21° C. The effect between 21 and 33° C, while less pronounced, still increases with increasing temperature. Temperature effect was also much more obvious in dry soil than either in moist mineral or

Fig. 1.
INFLUENCE OF SOIL TEMPERATURE ON THE BIOLOGICAL
ACTIVITY OF HEPTACHLOR



organic soil. By comparison, DDT has a negative temperature coefficient in soil (Fig. 2). It actually becomes more effective when the temperature decreases. In light mineral soil containing 12% water, for example, it was nearly twice as effective at 15°C as it was at 32°C. This factor was one of the great assets of DDT for cutworm control on tobacco. Treatments for control of the dark-sided cutworm were usually applied in late April or early May when soil temperatures tended to be low and it was under these conditions that DDT was most effective. DDT is one of the few insecticides that has a negative temperature coefficient. Nearly all others, at least all the organochlorine insecticides with the possible exception of lindane, have positive temperature coefficients. Heptachlor epoxide, technical chlordane and AG-chlordane also all have positive temperature coefficients in soil. To save time, I will not show you data which we have on the influence of soil and climatic factors on heptachlor epoxide, AG-chlordane or technical chlordane since the results which we have obtained closely parallel those which I have shown you with heptachlor.

Fig. 2.
INFLUENCE OF SOIL TEMPERATURE ON THE BIOLOGICAL ACTIVITY
OF DDT



In addition to the physical chemical characteristics of an insecticide and the factors which influence its biological activity in soil, it is also necessary to consider the effectiveness of each insecticide to each species of soil insect. Initially, the cyclodiene insecticides, particularly heptachlor, were remarkably effective against virtually all soil insects of economic importance. Beginning in the early 1950's, the cyclodiene insecticides received very extensive use in North America for soil insect control. By 1968, the problem of insecticide resistance began to show up (Table 4). Over the years we have accumulated baseline toxicity data on some of the species which have become resistant. Initially our main problems were with the seed-corn maggot, onion maggot and cabbage maggot. All of them became resistant between 15 to 20 generations to aldrin which was our main selection agent. In addition, we had cross-resistance to dieldrin, to heptachlor and to chlordane. Between 1958 and 1964, these three species of insects became resistant to all cyclodiene insecticides and thus, it was necessary to find alternative insecticides to control them. In addition to these three species of root maggots, in Canada we have other species that have become resistant more recently including the dark-sided and redbacked cutworms, the carrot weevil and also the carrot rust fly. The carrot weevil became resistant to the

Table 4. Development of Cyclodiene Insecticide Resistance by Soil Insects in Canada.

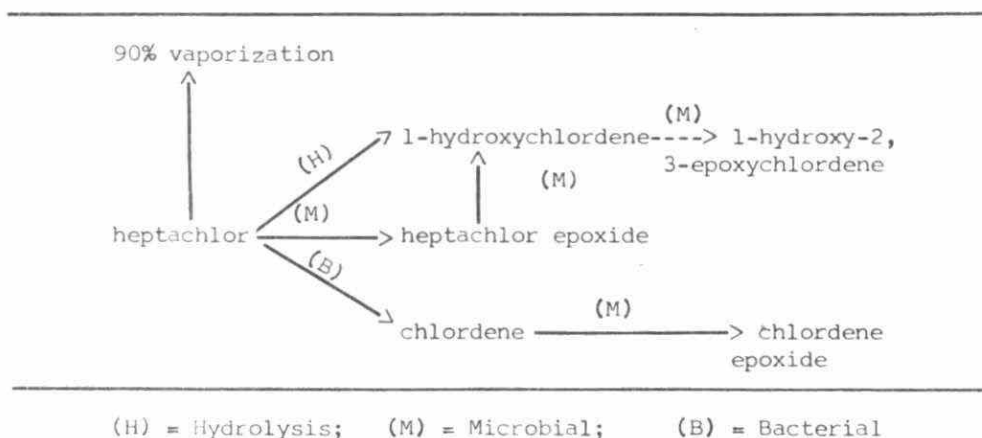
Insect	Level of resistance		
	aldrin	heptachlor	chlordan
<u>Root Maggots</u>			
seed-corn maggot	x 504	x 590	x 119
onion maggot	x 591	x 647	x 238
cabbage maggot	x 1127	x 1230	x 90
<u>Other species:</u> Canada - dark-sided and redbacked cutworms, carrot weevil, carrot rust fly.			
U.S. - wireworms, corn rootworm, grubs.			

cyclodiene insecticides in Ontario only in the last two years and you might wonder why it became resistant since we were no longer using cyclodiene insecticides. It would appear that the dieldrin and/or heptachlor epoxide residues in soil are still, in some instances, sufficiently high to cause selection pressure. Thus, we have the carrot weevil and some species of cutworms which are only now showing signs of becoming resistant to the cyclodiene insecticides. In the United States (Table 4) in addition to the species of insects which I have mentioned above, resistance to the cyclodiene insecticides has also occurred with some species of wireworms, the corn rootworm and grubs. Because of the resistance problem, many of the original recommendations which we made for the cyclodiene insecticides were dropped between 1958 and 1965. In Canada, after 1965 there were very few agricultural uses remaining for these materials, primarily for control of white grubs, wireworms and the northern corn rootworm.

When applied to soil, heptachlor itself is only a moderately persistent insecticide. Much of the heptachlor disappears from the soil within 2 or 3 years of the initial application by vaporization (Fig. 3).

Fig. 3.

DEGRADATION OF HEPTACHLOR IN SOIL



In addition, in moist soil, heptachlor will hydrolyse to 1-hydroxychlordene which is then metabolized by soil microorganisms to 1-hydroxy 2,3-epoxy-chlordene. Under certain conditions, heptachlor can also be metabolized by bacteria in soil to chlordene and by soil microorganisms to chlordene epoxide. However, a small proportion, perhaps 5 to 10% of the heptachlor is metabolized by soil microorganisms to heptachlor epoxide. Heptachlor epoxide is also an effective insecticide (Table 5), and in contact toxicity tests against crickets it is actually more effective than heptachlor.

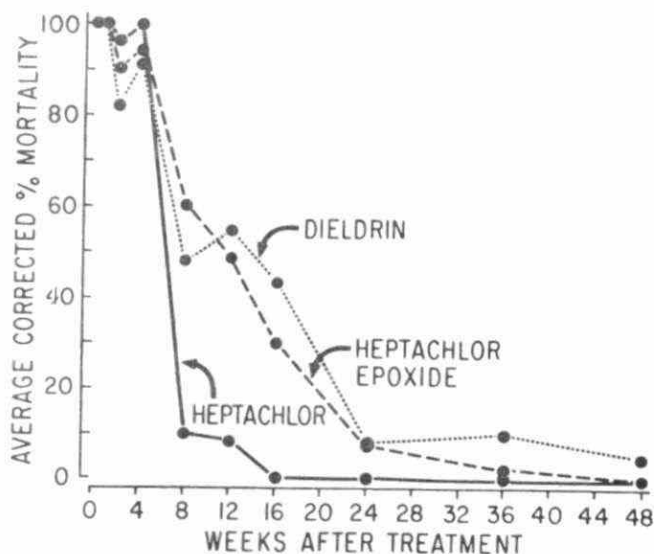
Table 5. Relative Toxicity of Heptachlor Epoxide to Crickets by Direct Contact and in Soil.

Insecticide	Av. corr. % mortality at indicated			
	% solution			
	0.001	0.01	0.1	1.0
heptachlor epoxide	0	75	100	100
heptachlor	0	0	100	100

	ppm in moist mineral soil			
	0.1	0.5	1	5
heptachlor	15	100	100	100
heptachlor epoxide	0	95	100	100

In soil, the reverse is the case and heptachlor is more effective than heptachlor epoxide. Nevertheless, heptachlor epoxide would be classed as a very effective soil insecticide. In our laboratory, one of our standard tests is to determine the persistence of biological activity of various insecticides in soil. The results shown in Fig. 4 provide a comparison of the rate at which the biological activity of heptachlor and heptachlor epoxide disappears from soil relative to dieldrin. These tests were done under controlled conditions of constant light, constant temperature of 80°F and 60±5% RH, so it is not a normal field condition. If you translate 48 weeks in the lab into field results, a compound persisting for a year in the lab is going to persist much longer in the field depending on the climatic conditions and the soil type. The data (Fig. 4) indicate that heptachlor is only a moderately persistent insecticide. After 16 weeks in the laboratory, all the biological activity disappeared. A small amount of heptachlor would have been converted to its epoxide, say 5%, but this small concentration would not be biologically active and we would not record any mortality. When we compare the results for heptachlor with heptachlor epoxide, you will notice that the epoxide persists for a much longer period of time with biological activity disappearing only after 48 weeks. Dieldrin appears to be a shade more persistent than heptachlor epoxide. Much has been said in the press about heptachlor being a persistent material. In actual fact, heptachlor is not a persistent material. A small amount of it is converted to the epoxide which is persistent but recent studies have also shown that this material is converted in soil by microorganisms to 1-hydroxychlordene.

Fig. 4.
RELATIVE PERSISTENCE OF BIOLOGICAL ACTIVITY
OF HEPTACHLOR, H.EPOXIDE AND DIELDRIN IN
MINERAL SOIL



We have been interested in heptachlor epoxide for a number of years and have done quite a few studies on it. One of the things that we have looked at is the movement down through soil (Table 6). These are the results of tests we did in the field at London where we treated the top 15 cm of soil

Table 6. Vertical Distribution of H. Epoxide (PPM) in Treated Soil ¹⁾ and Untreated Subsoil.

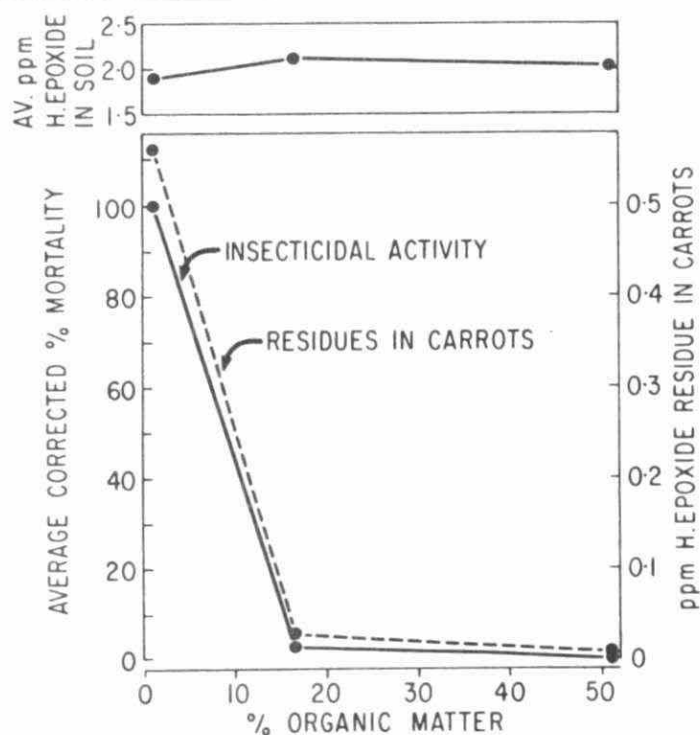
Strata	Sand		Muck	
	I ²⁾	F ³⁾	I	F
0-5	1.8	1.3	2.5	1.9
5-10	1.9	1.8	2.1	2.0
10-15	1.8	1.6	2.4	2.3
15-20	< 0.01	0.1	< 0.01	< 0.01
20-25	< .01	.04	< .01	< .01
25-30	< .01	< .01	< .01	< .01

- 1) Treated to a depth of 15 cm;
2) Initial sample;
3) 4 months after treatment.

with an emulsifiable concentrate of heptachlor epoxide provided through the courtesy of the Velsicol Corporation. Two soil types were used, sand and a muck. Both soils were treated at concentrations of approximately 2 ppm. Four months after treatment, there had been only a very small amount of degradation of the heptachlor epoxide in either the sand or muck soils. In addition, there had been minimal movement downward in the soil. In the light mineral soil 1/10 ppm of heptachlor epoxide was found at 15-20 cm depth with much smaller amounts occurring at the 20-25 cm depth and less than .01 ppm at the 25-30 cm depth. In the organic soil, there was no vertical movement downward below the initial treatment of 15 cm. In that same study, we looked at the biological activity of the heptachlor epoxide in soil throughout the growing season and we grew carrots in the soil which are a very good indicator crop to look at residues of the cyclodiene insecticides which will be picked up in relation to soil type. As I mentioned, the treatments were approximately 2 ppm and in addition to the sand and the muck soil, we included a 1:1 combination of sand and muck, (Fig. 5). Our laboratory studies have indicated that the major factor controlling biological activity in soil is organic matter. In this test the three soils contained from < 1% organic matter in the sand to 18% organic matter in the mixture of the 1:1 sand:muck and approximately 50% organic matter in the straight muck. There was very little decrease in heptachlor epoxide residues in any of the soil types throughout the growing season and average residues were approximately 2 ppm with all three soils. In the bioassay studies, we obtained 100% insecticidal toxicity in the sand at the various sampling periods throughout the growing season. In the soil that contained about 18% organic matter, we had very little biological activity and, in the soil containing 50% organic matter, we obtained no insecticidal activity.

Fig. 5.

INFLUENCE OF SOIL ORGANIC CONTENT ON INSECTICIDAL
ACTIVITY OF H.EPOXIDE AND ITS ABSORPTION BY CARROTS



The residues found in carrots paralleled the insecticidal toxicity exactly. We had high residues in the carrots grown in the sandy soil but they dropped off very markedly in the soil containing 18% organic matter and there was virtually no residue in the soil containing 50% organic matter. I think that this is a factor that many of our people involved in registration tend to ignore when they assess an insecticide. Often they tend to look at the chemical aspects of a compound only - how long does a chemical persist using chemical techniques, etc. when in fact we should be using both methods because both insecticidal activity and uptake by crops are dependent, not on the concentration of insecticide in the soil, but on the organic content in the soil. Many of our high residues of aldrin, dieldrin, heptachlor, or heptachlor epoxide are in vegetable-growing soils high in organic matter. To a very large extent they are inactivated and thus, are not insecticidally active or picked up by crops.

Technical chlordane is also an effective soil insecticide (Table 7). However, it is not nearly as toxic to insects as is heptachlor. As a contact poison against crickets it is approximately 1/10 as toxic as heptachlor and similar results were obtained when it was incorporated into moist mineral soil. In round figures we can estimate that technical chlordane is probably about 1/8 to 1/10 as active as heptachlor and this is because α - and γ -chlordane are not nearly as effective as heptachlor and, in technical chlordane, we are dealing primarily with a mixture of

Table 7. Relative Toxicity of T. Chlordane to Crickets by Direct Contact and in Soil

Insecticide	Av. corr. % mortality at indicated			
	% solution			
	0.001	0.01	0.1	1.0
heptachlor	0	22	100	100
t. chlordane	0	0	85	100
	ppm in moist mineral soil			
	0.1	0.5	1	5
heptachlor	20	100	100	100
t. chlordane	0	0	50	100

these three materials. Our analysis of technical chlordane (Table 8) indicates that it contains roughly equivalent amounts of γ -chlordane, α -chlordane and heptachlor. In one reference grade sample which we analyzed

Table 8. Insecticidal Components in T. Chlordane

Insecticide	%	
	Reference grade	Emulsifiable concentrate
γ -chlordane	10.5	11.6
α -chlordane	8.6	10.4
heptachlor	8.5 ¹⁾	9.4
nonachlor	ND ¹⁾	2.7
Totals	27.6	34.1

1) not determined

we found 10.5% γ -chlordane, 8.6% α -chlordane and 8.5% heptachlor, while in an emulsifiable concentrate, we found 11.6, 10.4 and 9.4% of these materials respectively. In addition, in the emulsifiable concentrate, we found 2.7% nonachlor. γ -chlordane, α -chlordane, and heptachlor comprise approximately 27-30% of technical chlordane. We have also looked at the biological activity of the insecticidal components of technical chlordane (Table 9). As mentioned earlier, technical chlordane is much less effective than heptachlor as a contact insecticide. γ -chlordane

Table 9. Relative Toxicity of Insecticidal Components of T. Chlordane to Crickets by Direct Contact and in Soil

Insecticide	Av. corr. % mortality at indicated			
	% solution			
	0.001	0.01	0.1	1.0
t. chlordane	0	0	85	100
heptachlor	0	22	100	100
γ -chlordane	0	0	100	100
α -chlordane	0	0	95	100
monachlor	0	0	56	100
	ppm in moist mineral soil			
	0.1	0.5	1	5
t. chlordane	0	0	50	100
heptachlor	20	100	100	100
γ -chlordane	0	0	50	100
α -chlordane	0	0	5	100
nonachlor	0	0	0	75

appears to be slightly more effective than technical chlordane, while α -chlordane is very similar in toxicity to technical chlordane. Nonachlor was again slightly less effective. In soil, the picture was similar, technical chlordane was approximately 1/8 to 1/10 as effective as heptachlor. γ -chlordane showed activity in soil equivalent to that obtained with technical chlordane, while both α -chlordane and nonachlor were less effective in soil than technical chlordane.

A point which has been of interest to us, is the extent to which the 8½% heptachlor content of technical chlordane contributes to the total insecticidal activity of technical chlordane. Based on some of the bioassay data which I showed you earlier, I have summarized results which we have obtained with technical chlordane, γ -chlordane, α -chlordane and heptachlor (Table 10). In light mineral soil, a concentration of 1 ppm of technical chlordane was required to cause cricket mortality. Based on the results of our chemical analysis 1 ppm of technical chlordane in that mineral soil would include .105 ppm of γ -chlordane, .086 ppm of α -chlordane and .085 ppm of heptachlor. However, bioassay studies have indicated that in the case of α - and γ -chlordane at least 1 ppm of these materials was required to cause insect mortality. However, heptachlor was 8 to 10 X as toxic as either α - or γ -chlordane and, as a result, 0.1 ppm of heptachlor was adequate to cause insect mortality. Referring to the data shown in Table 10, it is apparent that the .085 ppm of heptachlor, applied to soil in the treatment of 1 ppm of technical chlordane would be almost enough to result in insect mortality. By contrast, the concentrations of .105 and

Table 10. Insecticidal Activity of T. Chlordane in Relation to its Toxic Components.

Insecticide	% in t. chlordane	ppm components in soil treated with t. chlordane at 1 ppm	ppm in soil required to cause cricket mortality
t. chlordane	100.0	1.0	1.0
γ -chlordane	10.5	0.105	1.0
α -chlordane	8.6	.086	1.0
heptachlor	8.5	.085	0.1

The biological activity of t. chlordane is dependent on the joint action of its insecticidal components. However, heptachlor provides about 80% of the toxic action.

.086 ppm of γ -chlordane and α -chlordane respectively in that 1 ppm of technical chlordane would only be one tenth of the concentration required for these compounds to result in insect mortality. The conclusion that we have come to is that in technical chlordane the insecticidal activity of the α - and γ -chlordane is probably not too significant. By contrast the 10% heptachlor content of technical chlordane is sufficient to give approximately 85% of the activity and thus, it is our conclusion that heptachlor is the major insecticidal component of technical chlordane. I do agree with

Dr. Polen that the activity of technical chlordane may be, to some extent, due to the joint action of the components. I think, however, from data which we have obtained in the laboratory that this suggestion may apply more to the contact action of technical chlordane than to its activity in soil. When we apply the insecticide or its components to soil, we do not seem to get the same kind of joint action that we get when the insecticide is applied by direct contact. We have worked on this for several years, not only with heptachlor but with the other components and the results which we have obtained indicate that, while there is an additive effect, there does not appear to be a synergistic action. I do feel that heptachlor is responsible for the major portion of the activity of technical chlordane in soil.

We have also conducted laboratory studies on the persistence of biological activity of technical chlordane and its various components in soil. As in the previous data which I showed you with heptachlor and heptachlor epoxide we relate the persistence of biological activity to standard insecticides such as dieldrin, which is classed as a highly residual insecticide, aldrin, which is a moderately residual insecticide, and diazinon, as slightly residual. In the case of the slightly residual insecticides, biological activity will disappear in 4 to 8 weeks. In the case of the moderately residual insecticides, biological activity may persist for up to 16 weeks and in the case of the highly residual insecticides, biological activity will persist for 48 weeks or longer. In the case of technical chlordane and its components, we obtained some extremely interesting results (Fig. 6). Nonachlor, α -chlordane and γ -chlordane were all as persistent in soil as dieldrin, i.e. they were all highly persistent. Heptachlor, technical chlordane and chlordene were all moderately persistent. None of the compounds fell into the slightly residual category. It is very interesting to note that heptachlor and technical chlordane fell into the same category, i.e. moderately persistent. I would suggest that this is another result which would indicate that much of the activity of technical chlordane is resulting from the 8 to 10% heptachlor content. If α - and γ -chlordane were contributing significantly to the insecticidal activity then the persistence of biological activity of technical chlordane would have been longer, as indicated in Fig. 6 which shows that both α - and γ -chlordane are highly persistent insecticides.

AG-Chlordane could be considered as a "cleaned up" technical chlordane. Results of our analysis (Table 11). Indicate that in contrast to the present technical chlordane which contains roughly 10:10:10 of α - and γ -chlordane and heptachlor, AG-chlordane consists primarily of α - and γ -chlordane with heptachlor and nonachlor comprising less than 2% of the total. Data which we have obtained on the insecticidal activity of AG-chlordane (Table 12) indicates that, as a contact insecticide, it is less effective than either heptachlor or heptachlor epoxide. In addition, it appears that it is slightly less effective in soil than technical chlordane with the LD₅₀ for technical chlordane being 0.75 ppm and that of AG-chlordane as 1.29 ppm. (Fig. 7) This lower toxicity in soil can possibly be attributed to the fact that the main component of AG-chlordane is α -chlordane (68%) and earlier data on the activity of the components of technical chlordane in soil (Table 10) indicated that α -chlordane is less effective in soil than γ -chlordane. Thus, it might be necessary to use slightly increased rates for soil insecticide treatments if AG-chlordane were to replace technical chlordane. Nevertheless, AG-chlordane would be considered to be an effective soil insecticide.

Fig. 6.

PERSISTENCE OF BIOLOGICAL ACTIVITY OF
TECHNICAL CHLORDANE AND ITS INSECTICIDAL
COMPONENTS

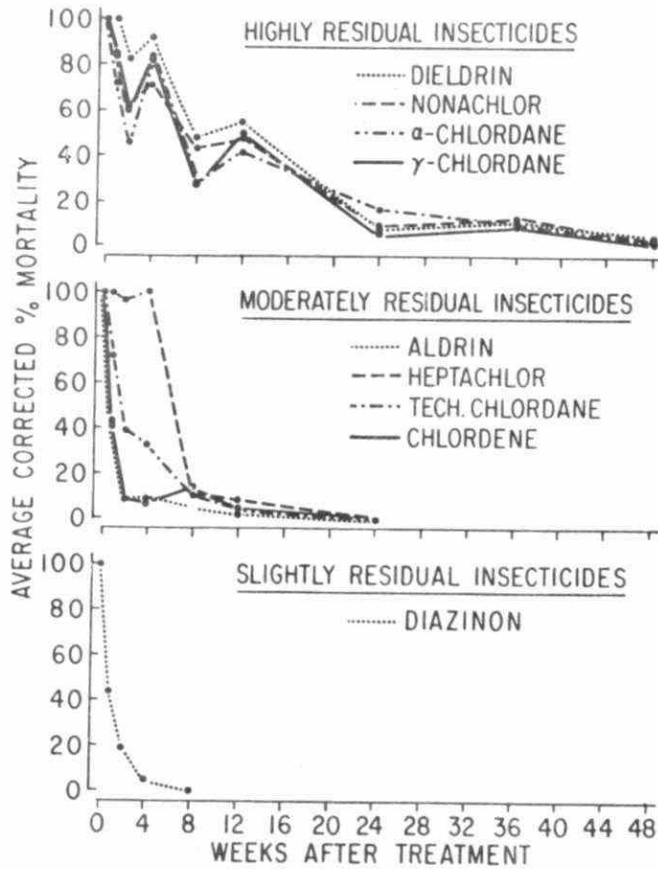


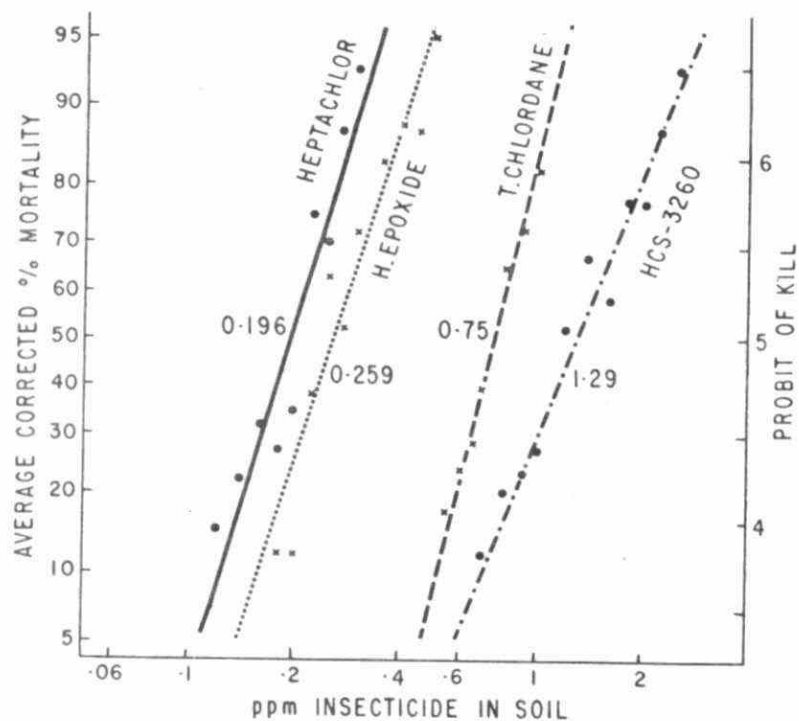
Table 11. Insecticidal Components in AG- as Compared to T. Chlordane

Insecticide	%	
	t. chlordane	AG chlordane
γ-chlordane	11.6	29.7
α-chlordane	10.4	68.0
heptachlor	9.4	1.0
nonachlor	2.7	0.6
Totals	34.1	99.3

Table 12. Relative Contact Toxicity to Crickets of AG-Chlordane, T. Chlordane, Heptachlor and H. Epoxide

Insecticide	Av. corr. % mortality at indicated % solution			
	0.001	0.01	0.1	1.0
H. epoxide	0	70	100	100
heptachlor	0	10	100	100
t. chlordane	0	0	100	100
AG-chlordane	0	0	100	100

Fig. 7.
TOXICITY IN SOIL OF AG-CHLORDANE, HEPTACHLOR,
H.EPOXIDE AND T.CHLORDANE TO CRICKETS



In assessing the behaviour of an insecticide in soil, one of the major criteria that we utilize is the extent to which it vaporizes. In the laboratory, the technique that we utilize is to test for fumigant toxicity by suspending test insects 1/4 inch above treated soil. The soil is treated at twice the dosage required to give us an LD₅₀ when the insects are exposed directly to the soil. In tests with heptachlor, technical chlordane, heptachlor epoxide and AG-chlordane (Table 13) we found that both heptachlor and technical chlordane were moderately volatile compounds. At a concentration

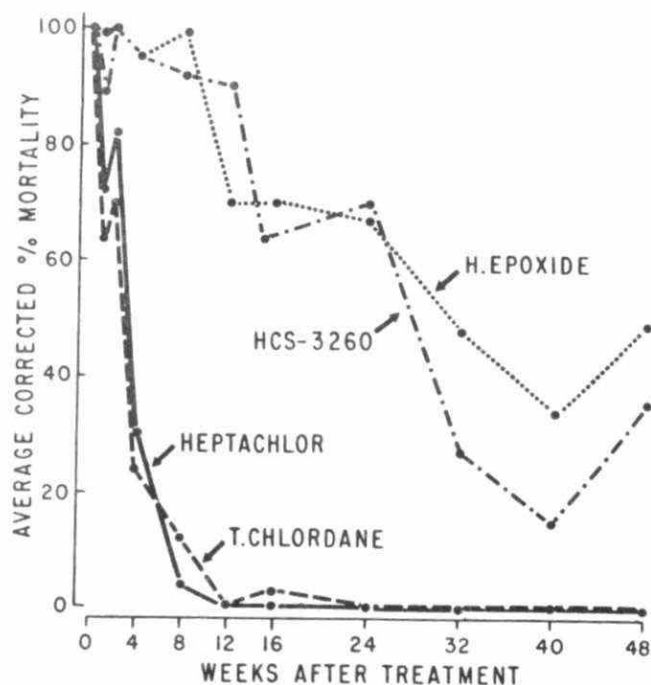
Table 13. Fumigant Toxicity of AG-Chlordane, T. Chlordane, Heptachlor and H. Epoxide in Moist Mineral Soil to Crickets.

Insecticide	ppm (2 x LD ₅₀) in soil	Av. corr. % mortality	ppm (4 x LD ₅₀) in soil	Av. corr. % mortality
heptachlor	0.4	75	0.8	95
t. chlordane	1.5	75	3.0	100
h. epoxide	0.5	0	1.0	20
AG-chlordane	2.6	0	5.2	9

of 2 X the LD₅₀ in soil, both heptachlor and technical chlordane cause 75% fumigant mortality. At a concentration of 4 X the LD₅₀ heptachlor caused 95% mortality and technical chlordane 100% mortality. In other words, much of the activity of heptachlor and technical chlordane in soil would be due to its fumigant activity. Heptachlor epoxide and AG-chlordane were much less volatile. At 2 X the LD₅₀ in soil, neither compound caused any fumigant toxicity. At 4 X the LD₅₀ heptachlor epoxide caused 20% mortality and AG-chlordane 9% mortality. Thus, heptachlor and technical chlordane fit into a category together, i.e. they are both moderately volatile, while AG-chlordane and heptachlor epoxide fit into another category, i.e. they are only slightly volatile. I think that this data is another argument for the contention that much of the activity of technical chlordane comes from the heptachlor in it. If α - and γ -chlordane were providing a significant amount of the insecticidal activity of technical chlordane, the fumigant toxicity of technical chlordane should have been lower than that of heptachlor. Our data have indicated that the behaviour of AG-chlordane in soil in relation to such factors as soil type, moisture and temperature, as well as the persistence of AG-chlordane in soil, closely parallels that of heptachlor epoxide. We have also looked at the persistence of biological activity of AG-chlordane in soil relative to heptachlor, heptachlor epoxide and technical chlordane (Fig. 8). You will note that the persistence of technical chlordane closely paralleled that of heptachlor, i.e. both materials were moderately persistent and biological activity disappeared within 16 weeks. In the case of heptachlor epoxide and AG-chlordane, they were both classed as highly persistent compounds in that, after 48 weeks under laboratory conditions, they still exhibited a significant degree of biological activity. It should be noted that these would be considered as minimum persistence values since the work was done in a light mineral soil where one would expect the insecticide to disappear moderately quickly.

Fig. 8.

PERSISTENCE OF BIOLOGICAL ACTIVITY OF
HEPTACHLOR, T.CHLORDANE, AG-CHLORDANE
AND H.EPOXIDE IN MINERAL SOIL



In summary, heptachlor is an excellent soil insecticide (Table 14). It is only moderately persistent but the formulated material contains approximately 20% γ -chlordane which can be classed as a highly persistent insecticide. Most heptachlor will vaporize from soil but small amounts are converted to heptachlor epoxide which is, in itself, both insecticidal and persistent. Both heptachlor and the epoxide will convert to 1-hydroxychlordene in soil and water. Heptachlor epoxide is absorbed by some crops in proportion

Table 14. Summary - HEPTACHLOR

1. Excellent soil insecticide; EC contains ca. 20% γ -chlordane.
2. Heptachlor is moderately persistent in soil; volatilizes; small amounts metabolized to heptachlor epoxide; both heptachlor and h. epoxide convert to 1-hydroxychlordene in soil and water.
3. H. epoxide is absorbed by some crops in proportion to concentration, organic content of soil, and climatic conditions.

to concentration and organic content of soil, as well as climatic conditions. Technical chlordane can be classified as a moderately good soil insecticide (Table 15). It is necessary to use it at considerably higher rates than those which we used when we were recommending heptachlor for soil insect control. There are four major insecticidal components in technical chlordane of which heptachlor provides most of the insecticidal action. The other three components, α - and γ -chlordane and nonachlor, are highly persistent in soil. AG-chlordane or HCS 3260 is also a moderately good soil insecticide which possibly will be slightly less effective in soil than the present technical chlordane. It contains four main insecticidal components of which α - and

Table 15. Summary - TECHNICAL CHLORDANE

1. Moderately good soil insecticide; contains ca. 20 components; 4 major insecticidal components are α -chlordane, γ -chlordane, heptachlor and nonachlor.
 2. Heptachlor provides most of the insecticidal action of technical chlordane.
 3. α -chlordane, γ -chlordane, and nonachlor are persistent in soil.
-

γ -chlordane comprise approximately 98%. Heptachlor and nonachlor comprise less than 2% of AG-chlordane. AG-chlordane will persist in soil and possibly will be as persistent as heptachlor epoxide and perhaps even dieldrin. Although less effective in soil its toxicity in relation to climatic factors, persistence and biological activity closely parallels that of heptachlor epoxide, (Table 16).

Table 16. Summary - AG-CHLORDANE (HCS-3260)

1. Moderately good soil insecticide; contains 4 insecticidal components - α - and γ -chlordane (ca. 98%), heptachlor and nonachlor (< 2%).
2. AG-chlordane is persistent in soil.
3. Although less effective, its toxicity in soil in relation to soil and climatic factors and persistence of biological activity closely parallels that of heptachlor epoxide.

CHEMICAL AND MICROBIAL DEGRADATION OF HEPTACHLOR AND HEPTACHLOR EPOXIDE

J. R. W. Miles

If insecticides are incorporated into soil which has been sterilized, the insecticides persist, relatively unchanged, just as though they were formulated on walnut shells or Attaclay. But when insecticides are incorporated into non-sterilized soils the insecticides are degraded, sometimes into numerous products, and this degradation has been attributed to the action of soil microorganisms. The usual products are oxidative. Two typical oxidation reactions occurring in soil are aldrin to dieldrin, and heptachlor to heptachlor epoxide (Fig. 1).

EPOXIDATION OF ALDRIN AND HEPTACHLOR

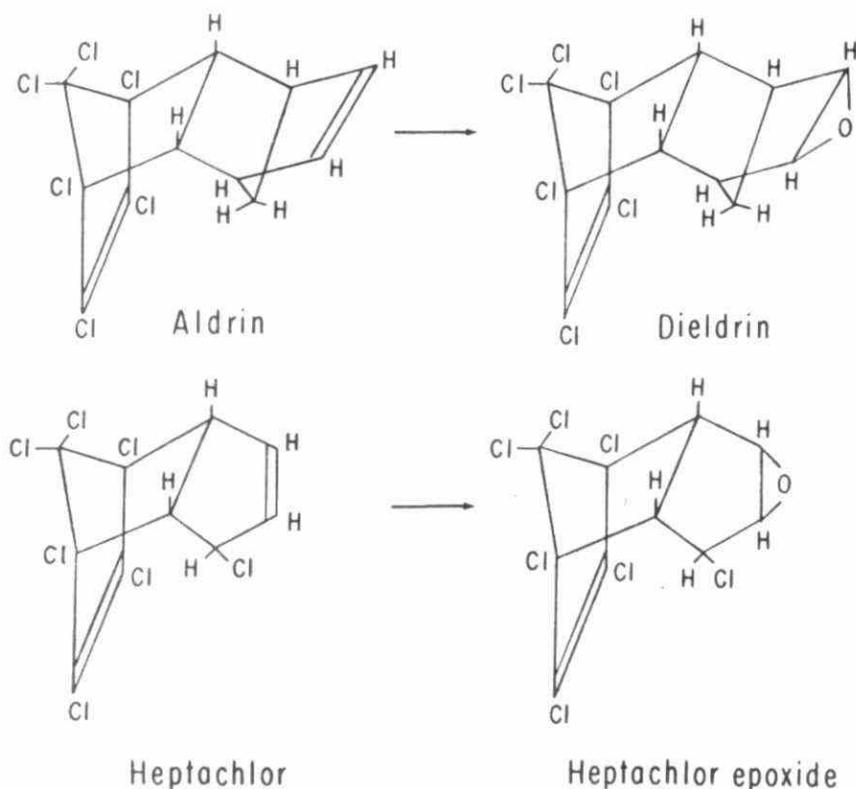


FIG. 1

When our laboratory acquired the services of a soil microbiologist, we began to investigate the action of soil microorganisms on insecticides.

Dr. Tu isolated 92 pure cultures from sandy loam soil of a farm near Guelph. We incubated these 92 microorganisms with aldrin, and determined the species which converted aldrin to dieldrin. We found only one product resulted from the aldrin incubations — dieldrin.

When we incubated these 92 microorganisms with heptachlor we found a number of products. But the main product, hydroxychlorde, was not the result of microbial action (Fig. 2). All our incubations were in 1% ethanolic medium.

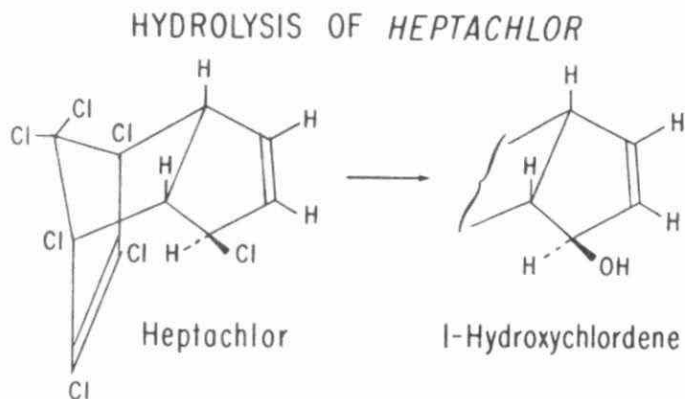
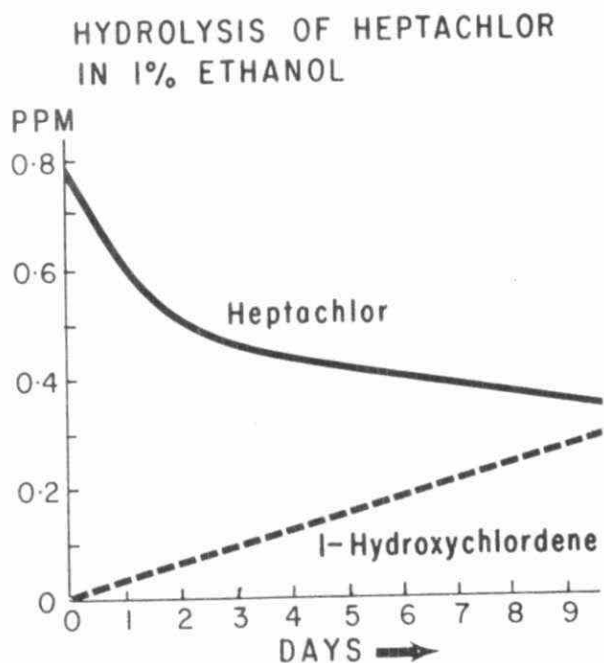


FIG. 2

And we found that heptachlor converted to hydroxychlorde in the alcoholic medium without any microorganisms being present (Fig. 3).



Heptachlor was also hydrolyzed to hydroxychlordene in distilled water (Fig. 4).

HYDROLYSIS OF HEPTACHLOR IN DISTILLED WATER

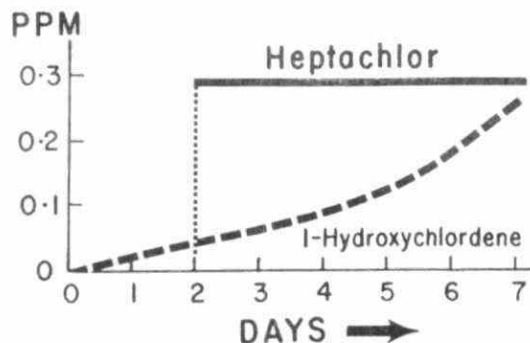
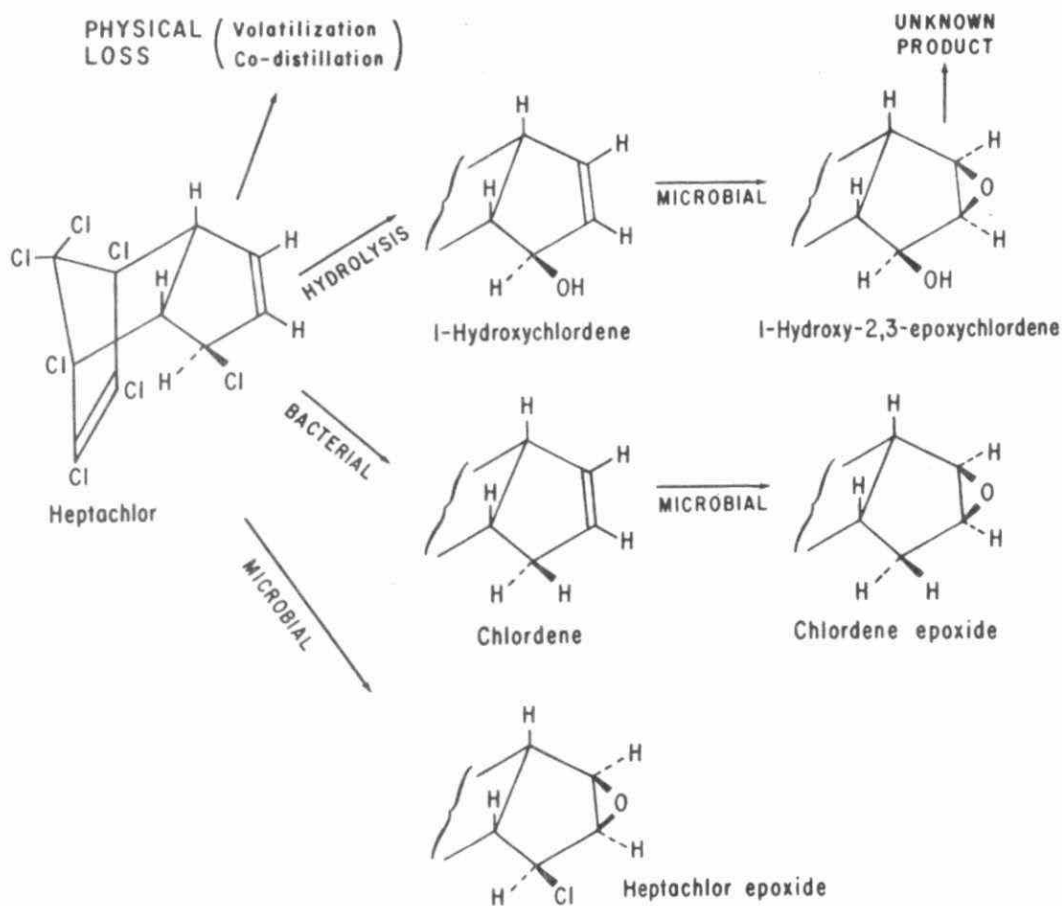


Fig. 4

All the products we found from incubating heptachlor with the soil microorganisms are shown in Fig. 5. There was a physical loss of heptachlor

SCHEME FOR CHEMICAL MICROBIAL DEGRADATION OF HEPTACHLOR



because we could not account for all the added heptachlor by the chemical and microbial degradation pathways. The main degradation route for heptachlor was by hydrolysis to hydroxychlordene. Conversion to hydroxychlordene amounted to 25-30% in the medium without microorganisms. Both bacteria and fungi were able to convert this hydroxychlordene to its epoxide, 1-hydroxy-2,3-epoxychlordene.

The reduction of heptachlor to chlordene had not been reported before. We found this reduction to occur only with bacteria, but the chlordene product was oxidized to chlordene epoxide by both bacteria and fungi.

The familiar oxidation of heptachlor to heptachlor epoxide was accomplished to various degrees by 35 of 47 fungi, and by 25 of 45 bacteria and actinomycetes.

But in soil the various microorganisms do not act alone as we have incubated them in this study. Synergism has been reported in the microbial degradation of diazinon - there probably is also antagonism in the mixed microbial population in the soil.

To examine the combined action of the microorganisms we extracted a mixed culture of soil microorganisms by shaking the sandy loam soil with distilled water and using the supernatant as our inoculant. When this mixed culture of microorganisms was incubated with heptachlor we did not obtain the epoxides as products until after 10 weeks' incubation. We did, however, get chemical hydrolysis to hydroxychlordene, and reduction to chlordene throughout the whole 12 weeks' incubation period.

The most important finding was when we incubated heptachlor epoxide with the mixed culture and obtained hydroxychlordene as the main product. This reaction had not been reported before. We theorize that under anaerobic conditions the heptachlor epoxide is reduced back to heptachlor which is then hydrolysed by moisture to hydroxychlordene. One substantiation of this theory was that in one of our flasks, along with 15% conversion to hydroxychlordene we had 5% chlordene. In this case the reduction must have carried on to

chlordene more rapidly than hydrolysis could occur. The conversion of heptachlor epoxide to hydroxychlordene averaged 1% per week, which is a significant rate of degradation, were it to occur in the field.

In our own experiments with heptachlor in soil we have not detected hydroxychlordene as a product. But Duffy and Wong in the Maritimes determined hydroxychlordene in amounts equal to or greater than the heptachlor epoxide concentration, in soils with a history of heptachlor treatment (Table 1).

Table 1. Residues (PPM) in Eastern Canadian Farm Soils, with History of Heptachlor Treatment ^{a)}

Farm	Heptachlor	Heptachlor epoxide	1-Hydroxy-chlordene	gamma chlordane
1	0.09	0.01	0.03	0.17
2	0.95	0.44	0.33	0.86
3	0.05	0.07	0.00	0.06
4	0.26	0.08	0.01	0.27
5	0.68	0.05	0.08	0.48

a) Soil sampled to 6 inch depth.

Ref: Duffy and Wong, J. Agr. Food Chem. 15, 457 (1967).

They also found considerable residues of gamma chlordane, presumably resulting from the gamma chlordane content of technical heptachlor.

In the United States, Carter and Stringer reported hydroxychlordene to be the main residue after soil treatment with heptachlor for termite control in Florida, Hawaii, Missouri and Oregon. (Table 2). Very high concentrations of hydroxychlordene were found in the Oregon soil presumably because of the heavy rainfall in that state. And again, there were very significant residues of gamma chlordane.

Table 2. Residues (PPM) in United States Soils 2 Years after Heptachlor Application a) b)

	Heptachlor	Heptachlor epoxide	1-Hydroxy- chlordene	gamma chlordane
Florida	88.1	1.0	6.8	32.5
Hawaii	9.6	6.5	1.2	7.6
Missouri	418.	-	-	124.
Oregon	21.2	-	90.0	43.8

a) water emulsion, 0.5% technical heptachlor, 1 pt/sq ft.

b) residues averaged down to 4 inch depth.

Ref: Carter and Stringer, J. Econ. Entomol. 63, 625 (1970).

What we have found then is a number of degradation pathways of heptachlor to much less toxic compounds, and also the degradation of heptachlor epoxide to hydroxychlordene.

Table 3 shows that acute oral LD₅₀ to rats of heptachlor, heptachlor epoxide, and hydroxychlordene. From this data it can be seen that the conversion

Table 3. ACUTE ORAL LD₅₀ TO RATS

Heptachlor	90-135 mg/kg
Heptachlor epoxide	60 mg/kg
1 Hydroxychlordene	2400 - > 4600 mg/kg

Ref: P. B. Polen, Velsicol Corp., Chicago,
Ill.

of either heptachlor or heptachlor epoxide to hydroxychlordene is a detoxification reaction and should be considered as an important factor in the assessment of heptachlor usage.

Soil and Crop Residues of High Purity Chlordane (Velsicol HCS-3260)
in British Columbia

by

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Field Plots, 20 by 20 feet, with 20 foot buffer strips separating the plots, were treated in duplicate with Velsicol HCS-3260 in June 1971 at 0, 5 and 10 pounds of actual ingredient per acre. One set of six plots (2 plots each at 0, 5 and 10 pounds) was set up on a farm near Langley in the Lower Fraser Valley in B.C. The soil of this field was free of any detectable residues prior to treatment with Chlordane. After staking out the plots, they were rototilled, raked and prepared for seeding. Then they were treated with HCS-3260 and immediately rototilled to the depth of 4 inches and raked smooth. Twenty-four hours after this treatment the soils of each plot were sampled. Then carrots, radishes and beans were seeded in each plot and potatoes planted. The edible portions of the crops were harvested when ready for marketing and then analyzed for residues of Chlordane and metabolites. The same crops were planted again in the same plots the following year (1972), and harvested and analysed as before. But the soils were not treated again with Chlordane in 1972.

Soil samples were collected 24 hours after treatment and then intermittently for 16 months.

The second set of 6 plots was set up in the Okanagan Valley on the CDA Research Farm in Summerland. In this case the plots were staked out in an established alfalfa field. Then the alfalfa was mowed to the ground using a rotary lawnmower at its lowest setting. Then the plots were raked clean. After treating these plots, the Chlordane was watered in thoroughly, not rototilled as in Langley. Soil samples were taken 24 hours after treatment. This soil also had been found to be free of detectable residues prior to treatment with Chlordane. Alfalfa was sampled and analysed 2, 4, 12, 13 and 15 months after treatment. It was cut and harvested routinely three months after treatment and again 12.5 and 14 months after treatment. Soils from these plots were sampled 0, 1, 3, 4, 12 and 15 months after treatment.

RESULTS AND DISCUSSION

Soils

Table 1 shows that the soil residues of Chlordane (α plus γ) were lower in Summerland than in Langley. This probably is due to the different application methods and the different climatic conditions. Figures 1 a,b and 2 a,b indicate the trend of the decline of the residues.

According to linear regression lines, calculated from all data, half lives for the 10-lb treatments are 9 and 10.5 months for Langley and Summerland, respectively. For the 5-lb treatments they are 13 1/2 and

14 1/2 months. Statistical determination of linearity, however, indicated that Summerland data are not sufficiently linear to permit conclusions, while linearity does obtain for Langley data - if initial residues (0 months) are not included (Fig. 3). Accordingly, half-lives for 10-lb and 5-lb treatments in Langley change to 11 and 6 months, respectively. So much for statistical high-jumps.

Of detectable Chlordane metabolites, only photo-cis-chlordane was found in Summerland soils ranging from traces to a maximum of 0.15 ppm which is a very small fraction of the total Chlordane residues in the soils. No metabolites were found in the Langley soils.

Crops

The interesting finding that the alfalfa contains considerable quantities of oxychlordane and of photo-cis-chlordane is discussed in detail in the attached manuscript entitled "Residues in Alfalfa following soil Treatment with High Purity Chlordane". Figures 4 and 5 also show these results. The demonstration that oxychlordane occurs in alfalfa grown in soils treated with HCS-3260 was confirmed in 1972 in a new experiment in the same location (Summerland) where two new plots were treated as in 1971 and again oxychlordane was found in the alfalfa. In no case could oxychlordane be found in the corresponding soils.

Table 2 Figures 6 and 10 show the Chlordane residues in the crops grown in the Lower Fraser Valley. In the 10-lb plots. The residue levels were: radishes > potatoes ≥ carrots >>> beans, ranging from a high of 0.12 ppm for radishes to a low of 0.008 ppm for beans (Fig. 10). Grown in the

same plots (i.e., those treated in 1971) during the following year, the residues in radishes were considerably lower but not much lower in potatoes and carrots (Fig. 6 to 8, 10). Apparently, weathering of residues of Chlordane makes these less available to radishes but not much less available to potatoes and carrots.

In the 5-lb treatments, potatoes showed the highest residues in 1971 closely followed by radishes and carrots. In all cases (5-lb plots) were the residues much lower in 1972 as compared to 1971, indicating that the residues picked up by these crops from soils after low-rate application become progressively less available as compared to residues from high-rate applications.

In potatoes, chlordane concentrations in peel and pulp were compared, and the ratios, show in Table 2, indicate much higher concentrations in the peel. Since the ratio of the weight of peel over the weight of pulp differs between samples (some housewives are greedier than others!), the data are expressed in Chlordane found in one tissue per total sample weight i.e., per weight of peel plus weight of pulp. The actual concentrations (ppm) in each tissue are given in brackets and emphasize the great difference.

The residues in beans were very low in both 10-lb and 5-lb plots, namely 0.0066 ppm and 0.0032 ppm, respectively, in 1971 (Fig. 9,10). Interestingly, however, these residues were the same during the following year, namely in 1972, i.e., unlike the root crops, beans picked up as much one year after application as they did the year of application.

From our results, the following conclusions and salient points are indicated regarding vegetables grown in soils treated with HCS-3260

(Fig. 10):

1. The magnitude of the residues depends on the rate of application and is roughly twice as high following application of 10-lb of Chlordane as it is following a 5-lb application.
2. Edible portions of root crops contain much more residues than green bean pods.
3. Residues in beans, although low, are the same if grown one year after application as they are if grown immediately following treatment, regardless of application rate.
4. In root crops grown in 5-lb plots one year after application, residues are lower by almost one half (approximately 45%) than they are in the same crops grown immediately after application.
5. In carrots and potatoes grown in 10-lb plots, however, the decline from one year to the next is only 15 to 20%, while it is approximately 50% in radishes.
6. The concentrations are much higher in peels of potatoes than in the pulp of the same tubers.
7. No metabolites of cis- or trans-chlordane (such as 1,2-dichloro-chlordene, oxychlordane, or photo-cis-chlordane) are found in these vegetables.

Table 1. Soil Residues from HCS-3260 in p.p.m. of dry weight^{a/}

	Months	Langley, B.C. ^{c/}			Months	Summerland, B.C. ^{d/}		
		α	γ	$\alpha + \gamma$		α	γ	$\alpha + \gamma$
10 lbs/acre	0 ^{e/}	5.379 (100) ^{b/}	1.911 (100) ^{b/}	7.290	0	2.801 (100)	1.055 (100)	3.856
	1	3.930 (73)	1.379 (72)	5.309	1	3.134 (112)	1.024 (97)	4.158
	2	3.910 (73)	1.516 (79)	5.426	2			
	3	3.127 (58)	1.171 (61)	4.298	3	2.025 (72)	0.704 (67)	2.729
	4	3.808 (71)	1.378 (72)	5.186	4	1.578 (56)	0.577 (55)	2.155
	12	2.548 (47)	0.857 (45)	3.405	12	2.511 (90)	0.836 (79)	3.347
	14	2.347 (44)	0.828 (43)	3.175	14			
	15				15	2.061	0.638	2.699
	16	2.324	0.707	3.030	16			
5 lbs/acre	0 ^{e/}	3.021 (100)	1.113 (100)	4.134	0	1.190 (100)	0.441 (100)	1.631
	1	1.435 (48)	0.529 (48)	1.964	1	1.228 (103)	0.482 (109)	1.710
	2	1.666 (55)	0.611 (55)	2.277	2			
	3	1.694 (56)	0.633 (57)	2.327	3	0.969 (81)	0.379 (86)	1.348
	4	1.698 (56)	0.587 (53)	2.285	4	0.630 (53)	0.237 (54)	0.867
	12	0.959 (32)	0.324 (29)	1.283	12	0.806 (68)	0.253 (57)	1.059
	14	1.154 (38)	0.410 (37)	1.564	14			
	15				15	0.644	0.216	0.860
	16	0.933	0.317	1.250	16			

^{a/} Each result is mean of 4 analyses (2 analyses per plot).

^{b/} Figures in brackets are % of initial.

^{c/} HCS-3260 applied in June 1971 and rototilled to ~ 4".

^{d/} HCS-3260 applied in June 1971 and watered in.

^{e/} Sampled 24 hrs after application.

Table 2. HCS-3260 residues in edible portions of crops (p.p.m. of fresh weight)
after soil treatments at 5 and 10 lbs/acre in June 1971.

Grown (Year)	Crop	Growing Time (Days)	10 lbs/acre			5 lbs/acre		
			α	γ	$\alpha + \gamma$	α	γ	$\alpha + \gamma$
1971	Carrots (Scarlet Nantes)	120	0.0632	0.0203	0.0835	0.0295	0.0098	0.0393
1972		112	0.0493	0.0156	0.0649	0.0159	0.0058	0.0217
1971	Potatoes (Netted Gem)	131	0.0679	0.0208	0.0887	0.0486	0.0146	0.0632
1972		112	0.0602	0.0168	0.0770	0.0270	0.0083	0.0353
1971	Beans (Top Crop)	82	0.0052	0.0014	0.0066	0.0023	0.0009	0.0032
1972		77	0.0057	0.0020	0.0077	0.0023	0.0011	0.0034
1971	Radishes (Cherry- belle)	40	0.0907	0.0324	0.1231	0.0430	0.0138	0.0568
1972		39	0.0510	0.0152	0.0662	0.0262	0.0054	0.0316

Peel vs. Pulp in Potatoes:^{a/}

lbs/acre	1971			1972		
	PEEL	PULP	RATIO	PEEL	PULP	RATIO
5	0.101 (0.672)	0.009 (0.014)	11 (48)	0.023 (0.249)	0.004 (0.006)	6 (42)
10	0.168 (0.731)	0.022 (0.021)	8 (35)	0.077 (0.868)	0.017 (0.017)	5 (51)

^{a/} μ g of chlordane in tissue per gramme of total potato sample, i.e., pulp plus peel. Figures in brackets ppm in the particular tissue.

Figure 1a. Persistence of Velsicol HCS-3260 in a Berry Series
Silty clay loam - Langley, B.C. (10 lbs/acre)

⊙ $\alpha + \gamma$ - Chlordane

△ α - Chlordane

□ γ - Chlordane

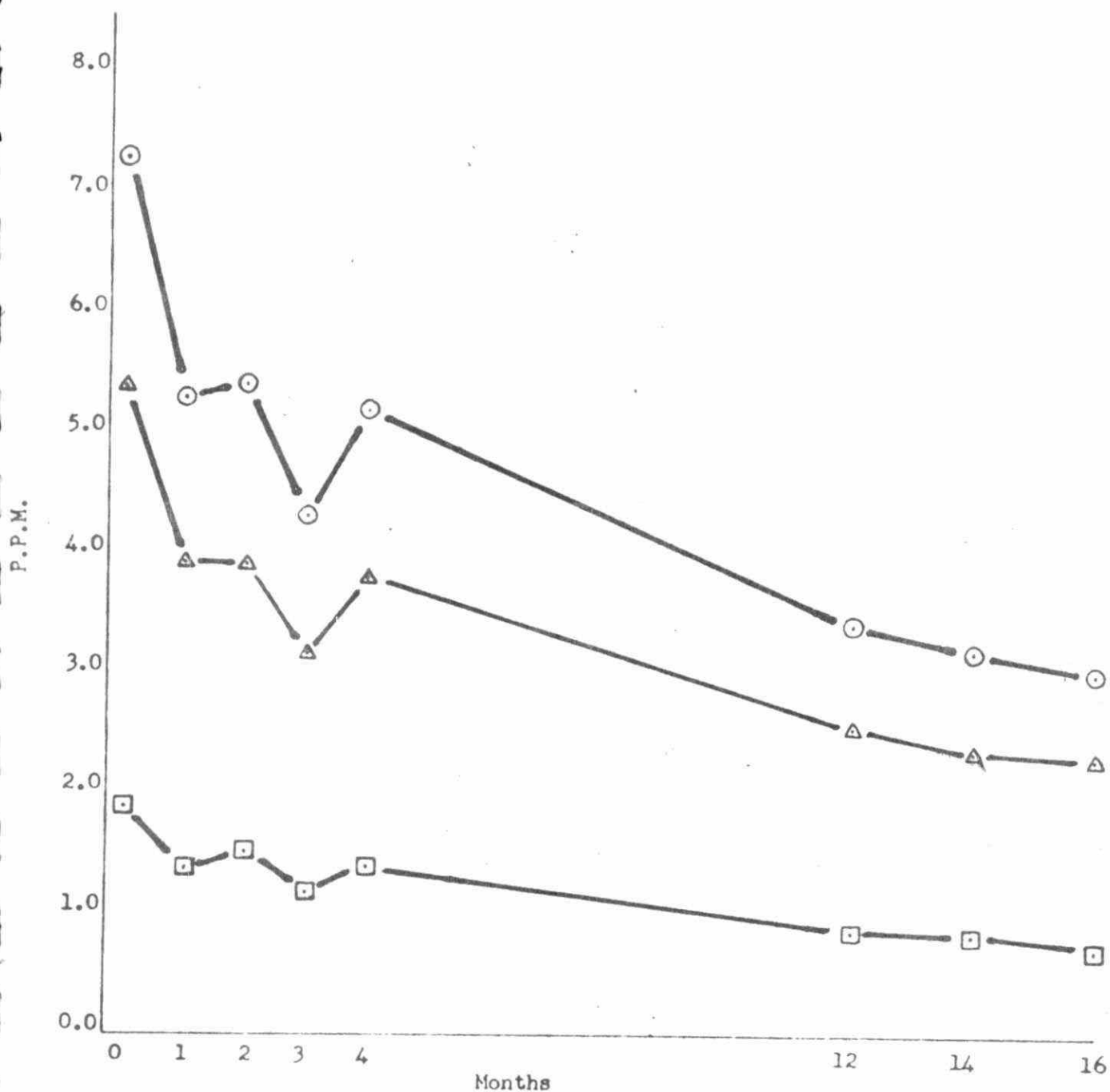


Figure 1b. Persistence of Velsicol HCS-3260 in a Berry Series
silty clay loam - Langley, B.C. (5 lbs/acre)

- ⊙ $\alpha + \gamma$ - Chlordane
- △ α - Chlordane
- γ - Chlordane

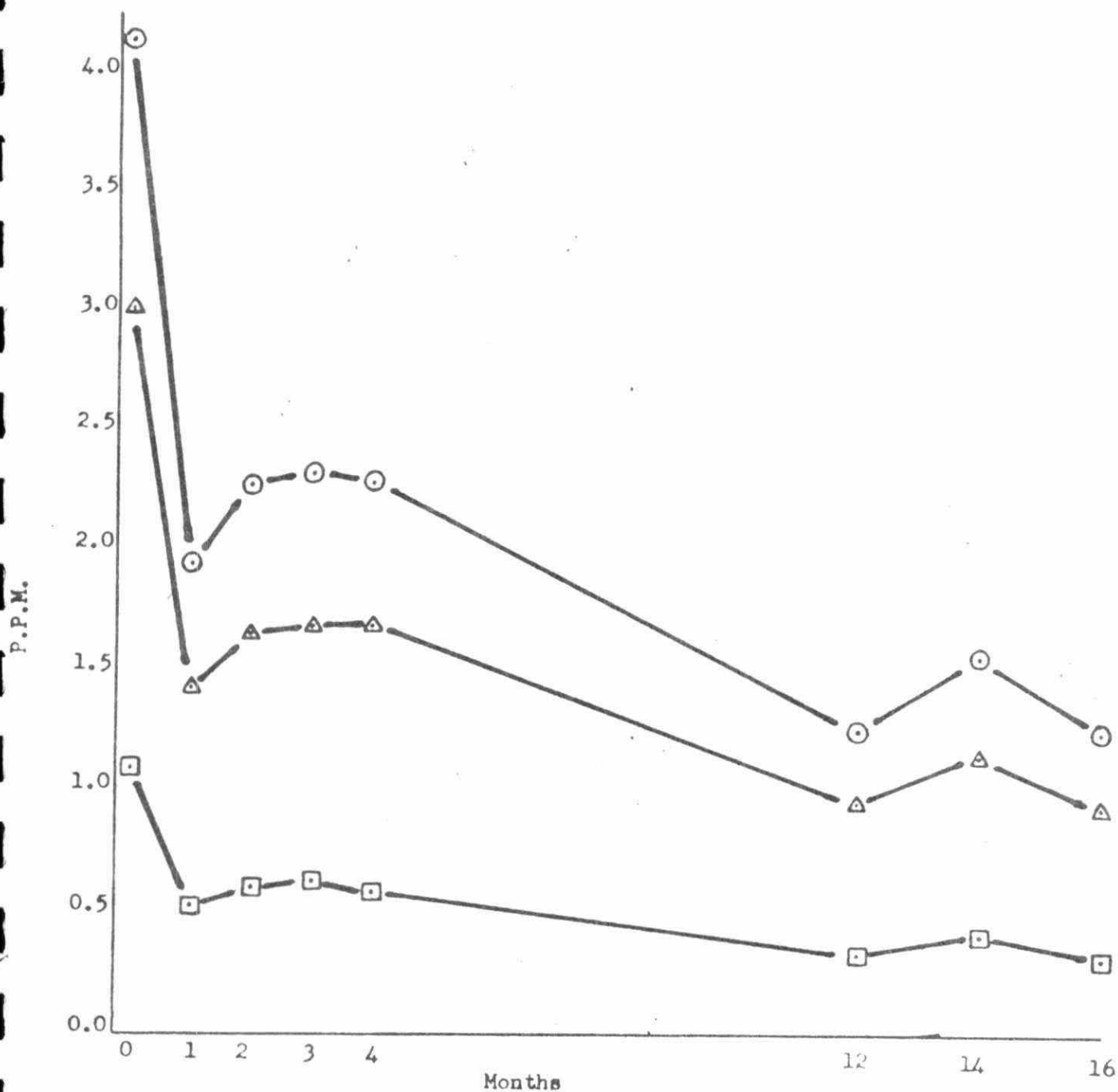


Figure 2a. Persistence of Velsicol HCS-3260 in an Osoyoos Series sandy loam - Summerland, B.C. (10 lbs/acre)

- ⊙ $\alpha + \gamma$ - Chlordane
- △ α - Chlordane
- γ - Chlordane

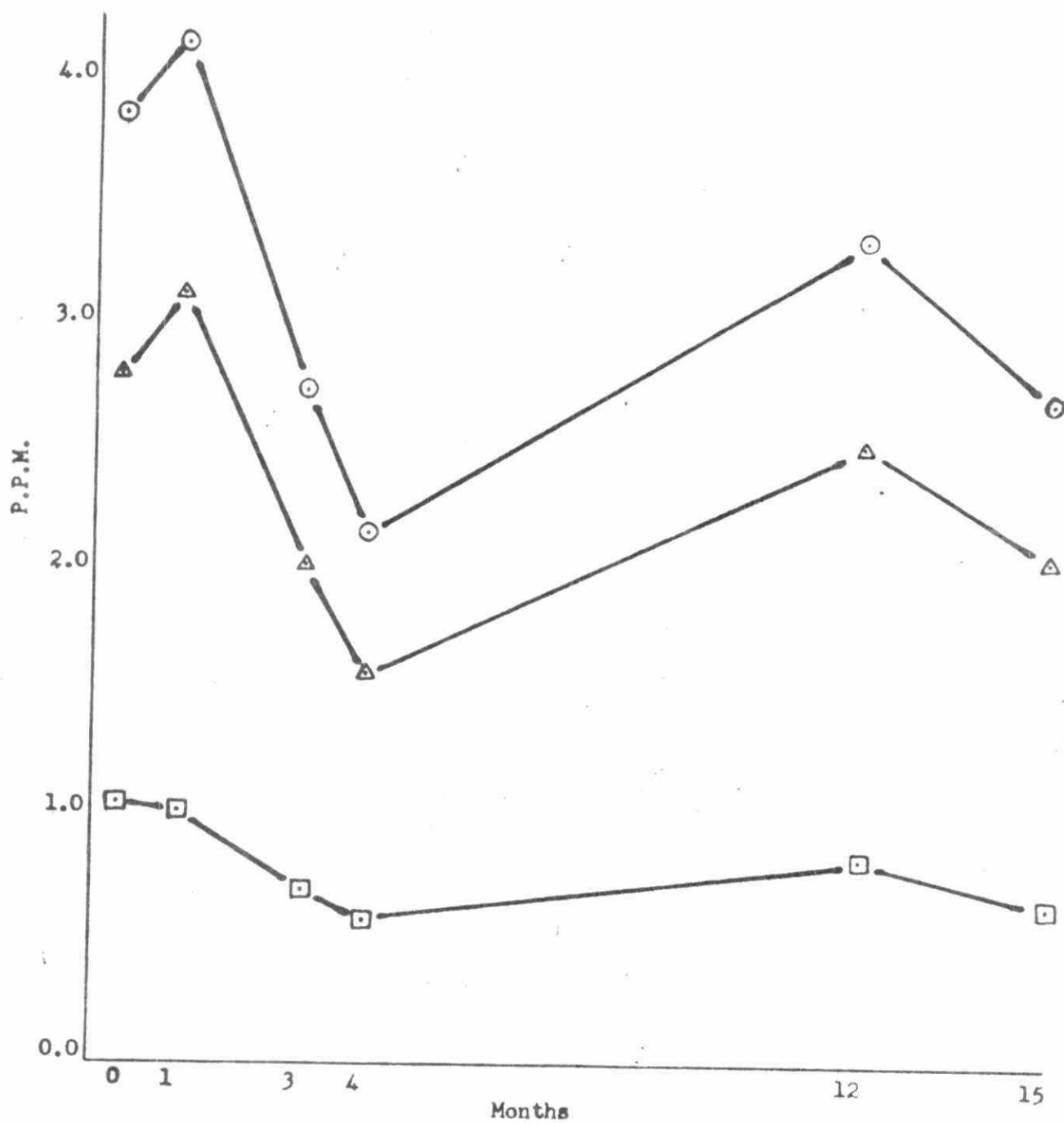


Figure 2b. Persistence of Velsicol HCS-3260 in an Osoyoos Series sandy loam - Summerland, B.C. (5 lbs/acre)

○ $\gamma + \alpha$ - Chlordane
 △ α - Chlordane
 □ γ - Chlordane

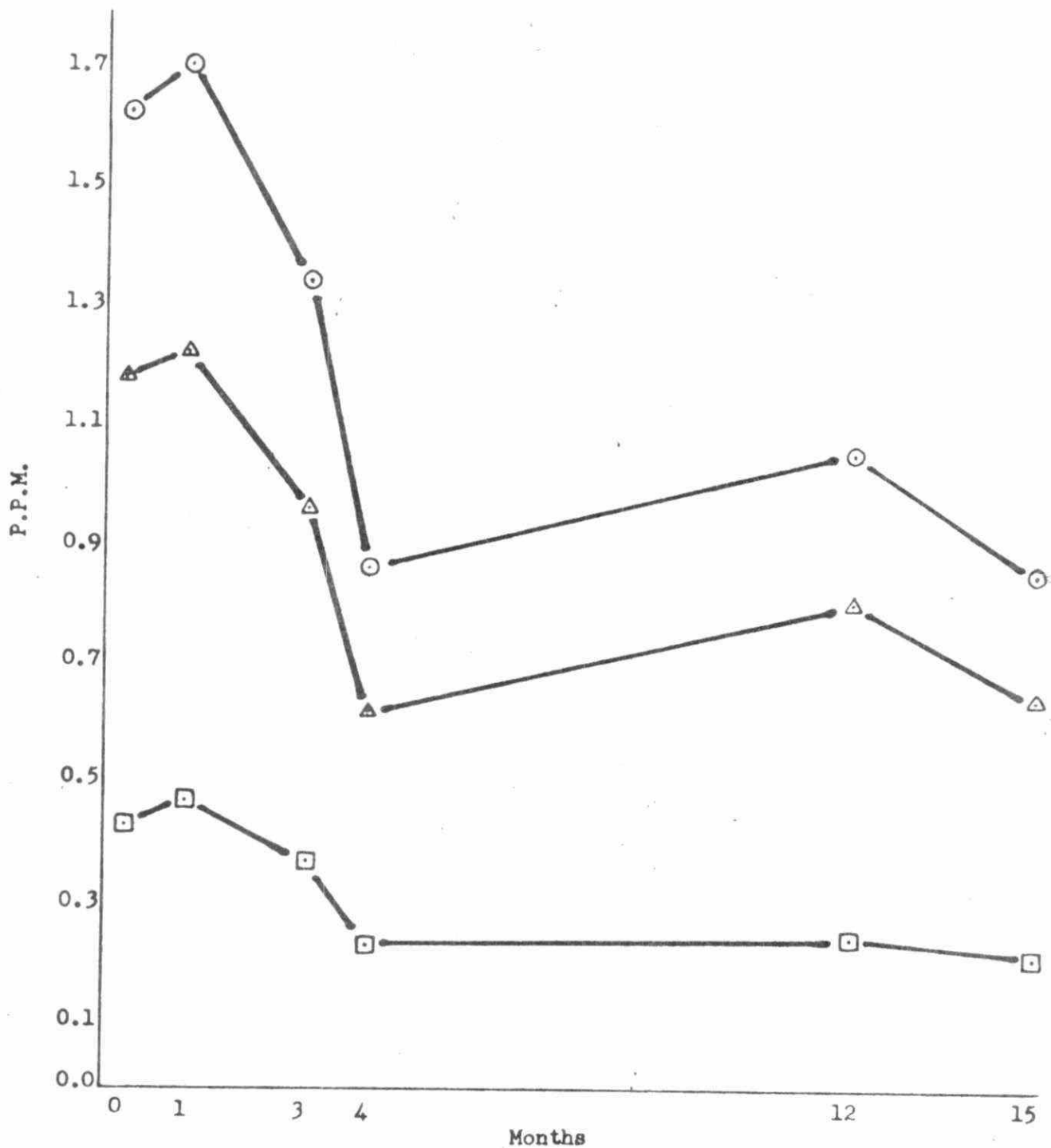


Figure 3. Calculated Regression Lines and 95% Confidence Limits for Velsicol HCS-3260 Persistence in Langley Soil

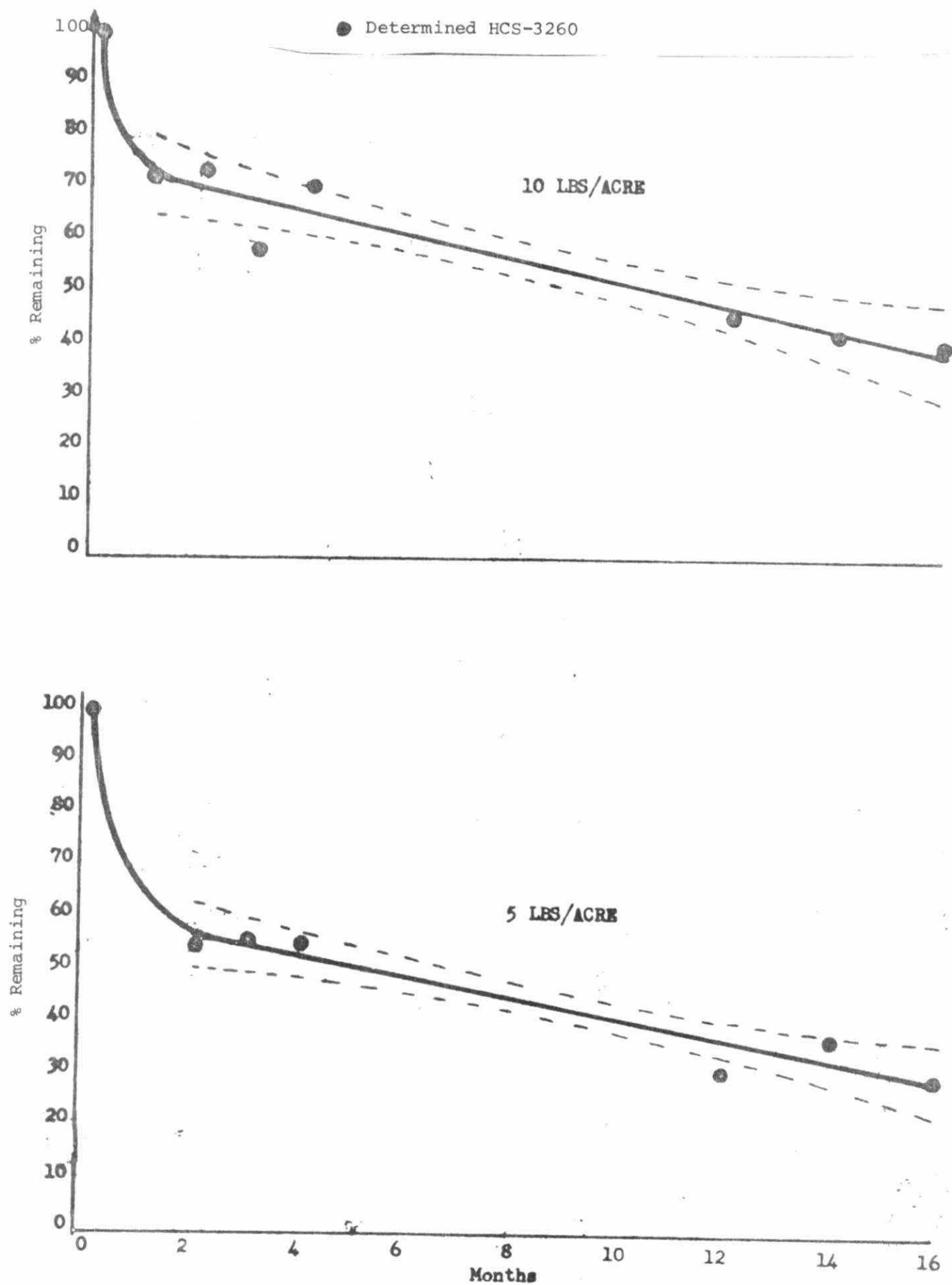


Figure 4. Alfalfa Residues from HCS-3260 in P.P.M. of Fresh Weight - Summerland, B.C. (10 lbs./acre)

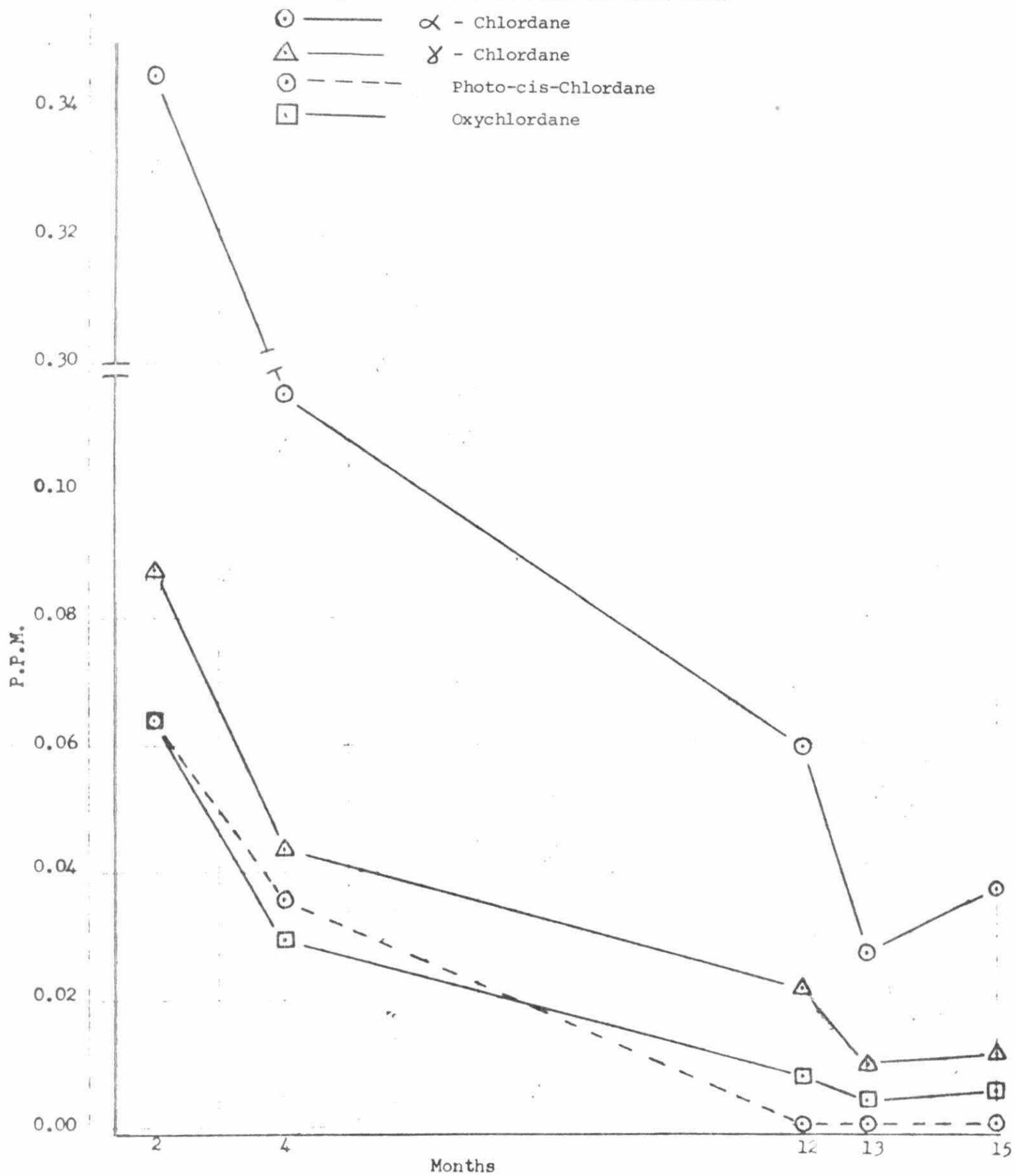


Figure 5. Alfalfa Residues from HCS-3260 in P.P.M. of Fresh Weight - Summerland, B.C. (5 lbs/acre)

○ ——— α - Chlordane
 △ ——— γ - Chlordane
 ⊙ - - - Photo-cis-Chlordane
 □ ——— Oxychlordane

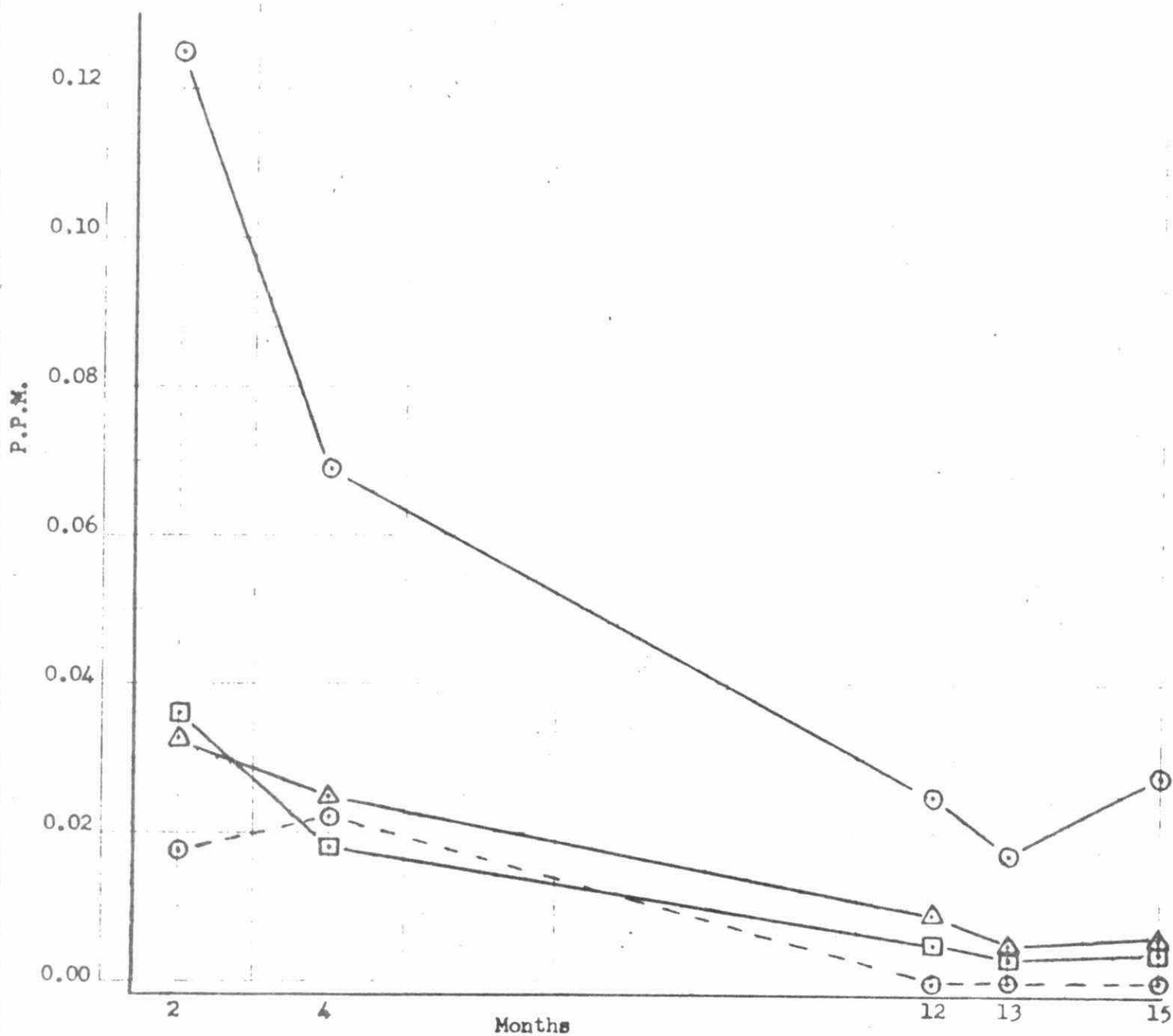


Figure 6. Radish Residues from HCS-3260 in P.P.M. of
Fresh Weight - Langley, B.C.

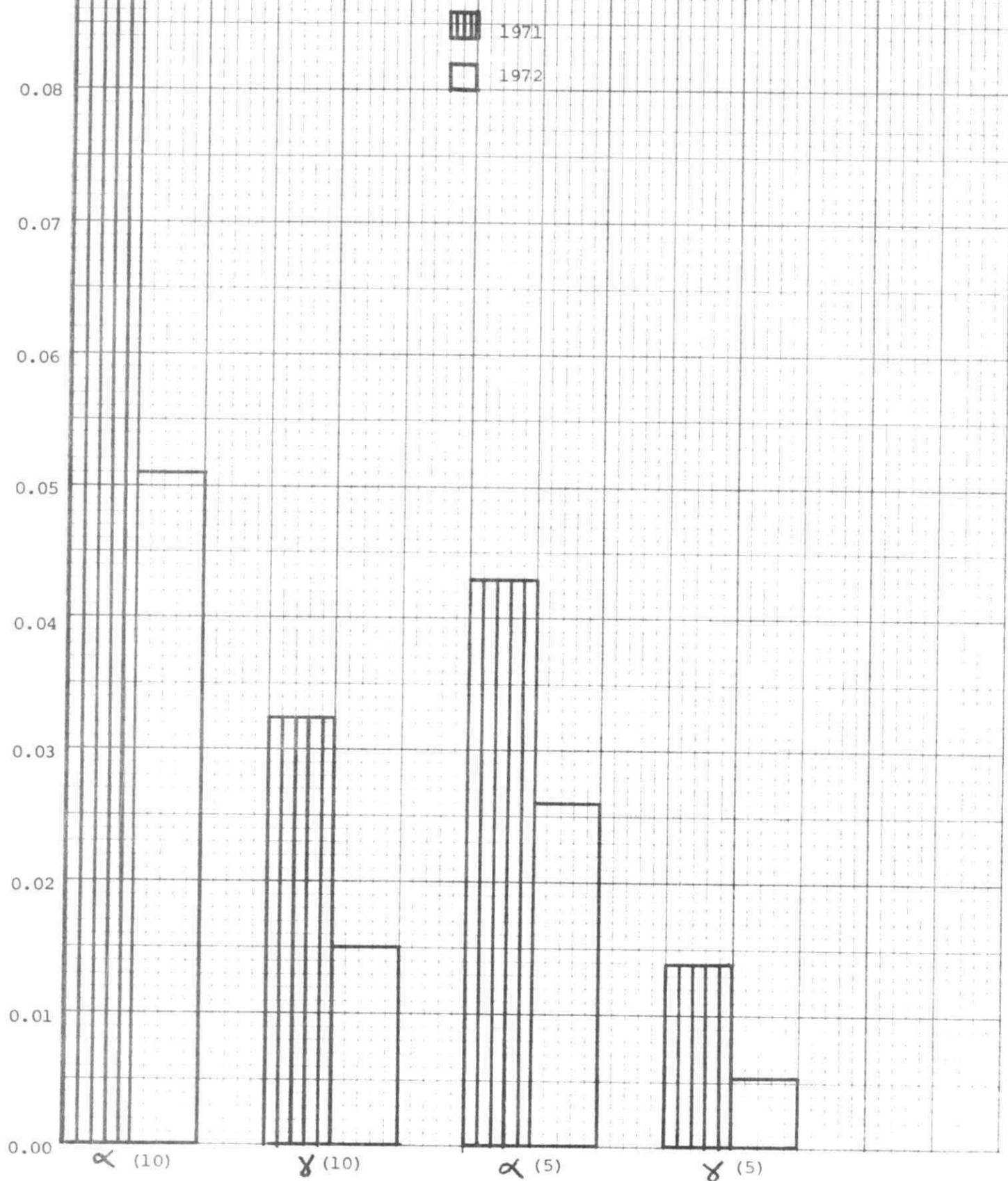


Figure 7. Potato Residues from HCS-3260 in P.P.M. of
Fresh Weight - Langley, B.C.

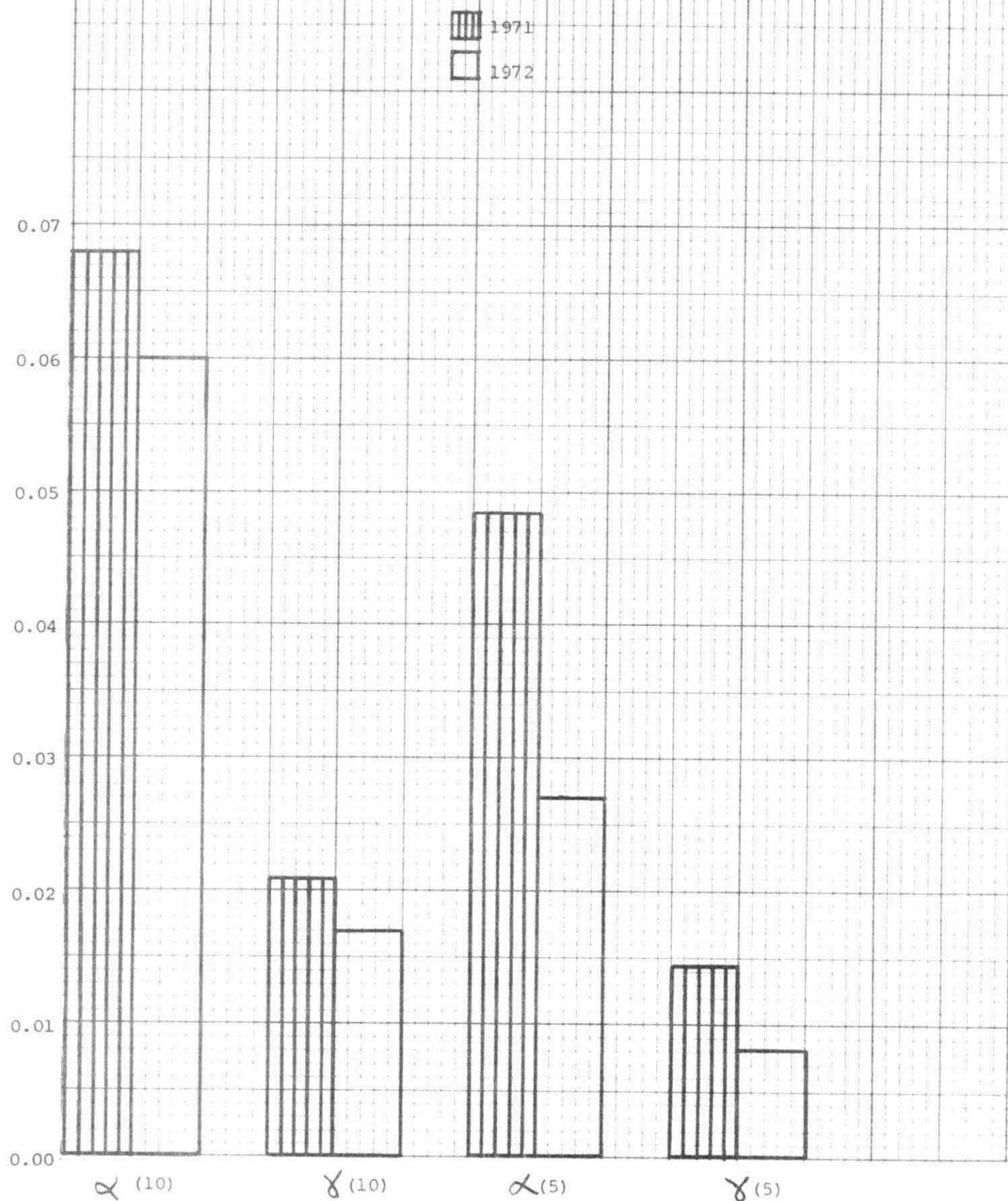


Figure 8. Carrot Residues from HCS-3260 in P.P.M. of
Fresh Weight - Langley, B.C.

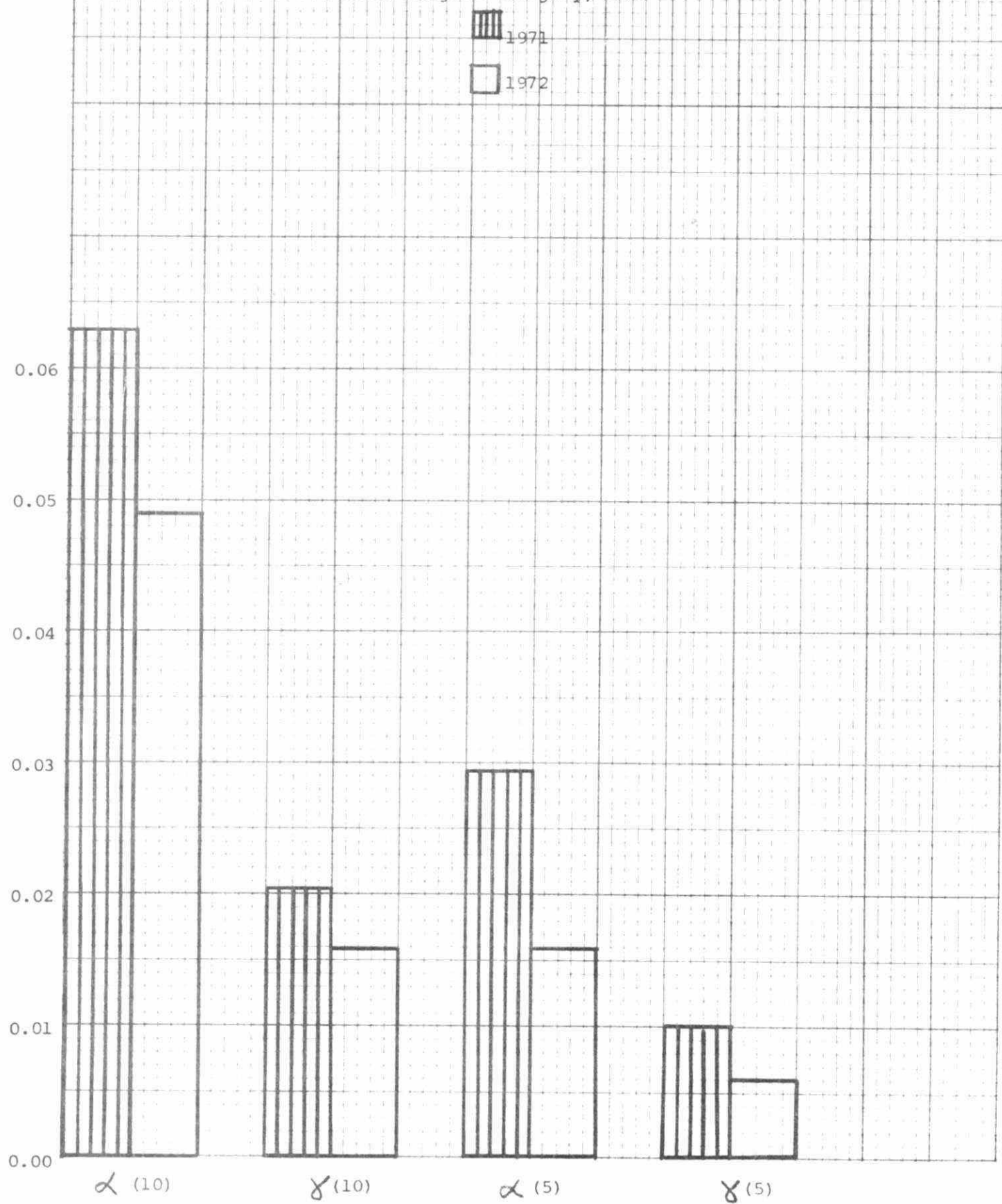


Figure 9. Bean Residues from HCS-3260 in P.P.M. of
Fresh Weight - Langley, B.C.

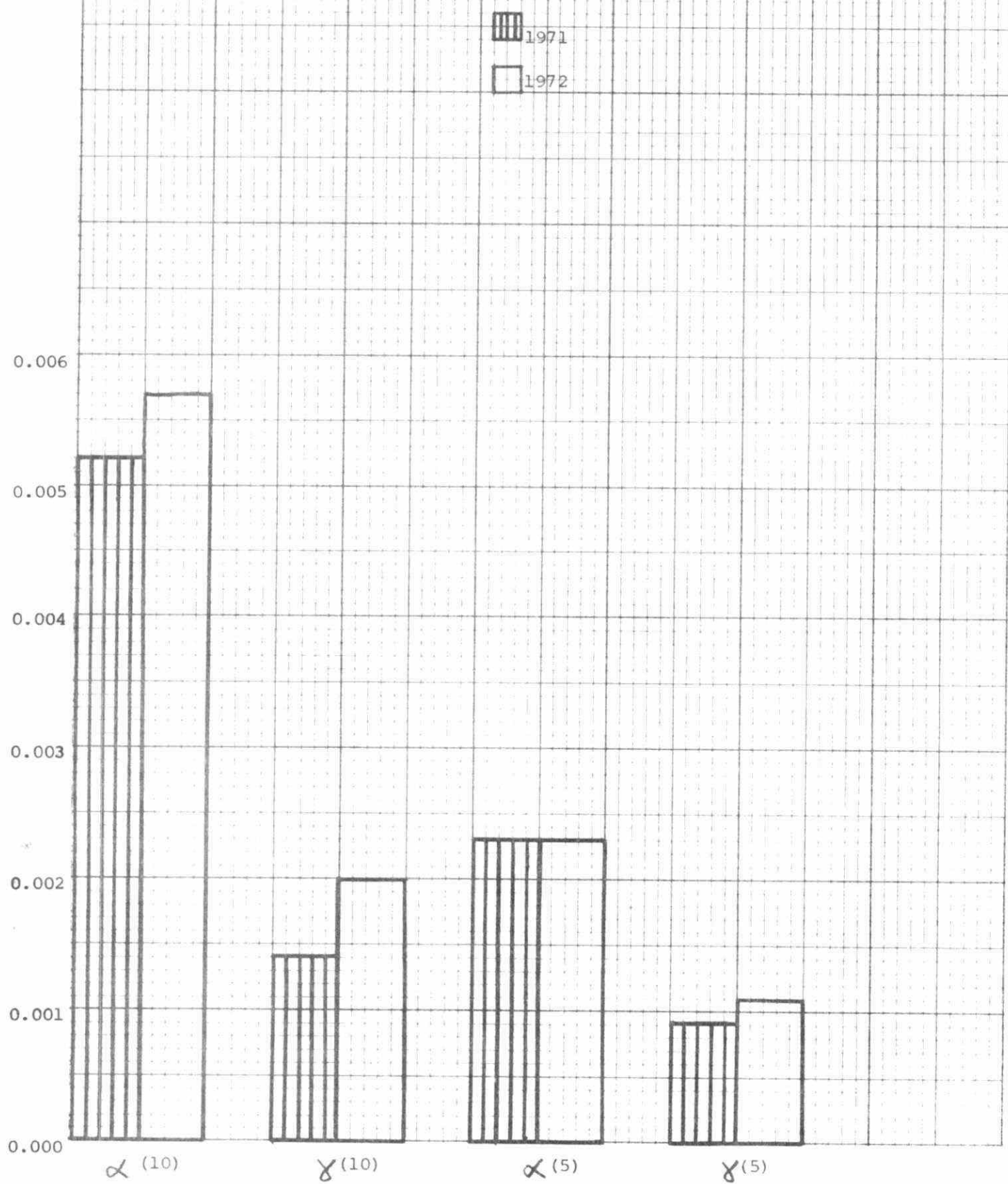
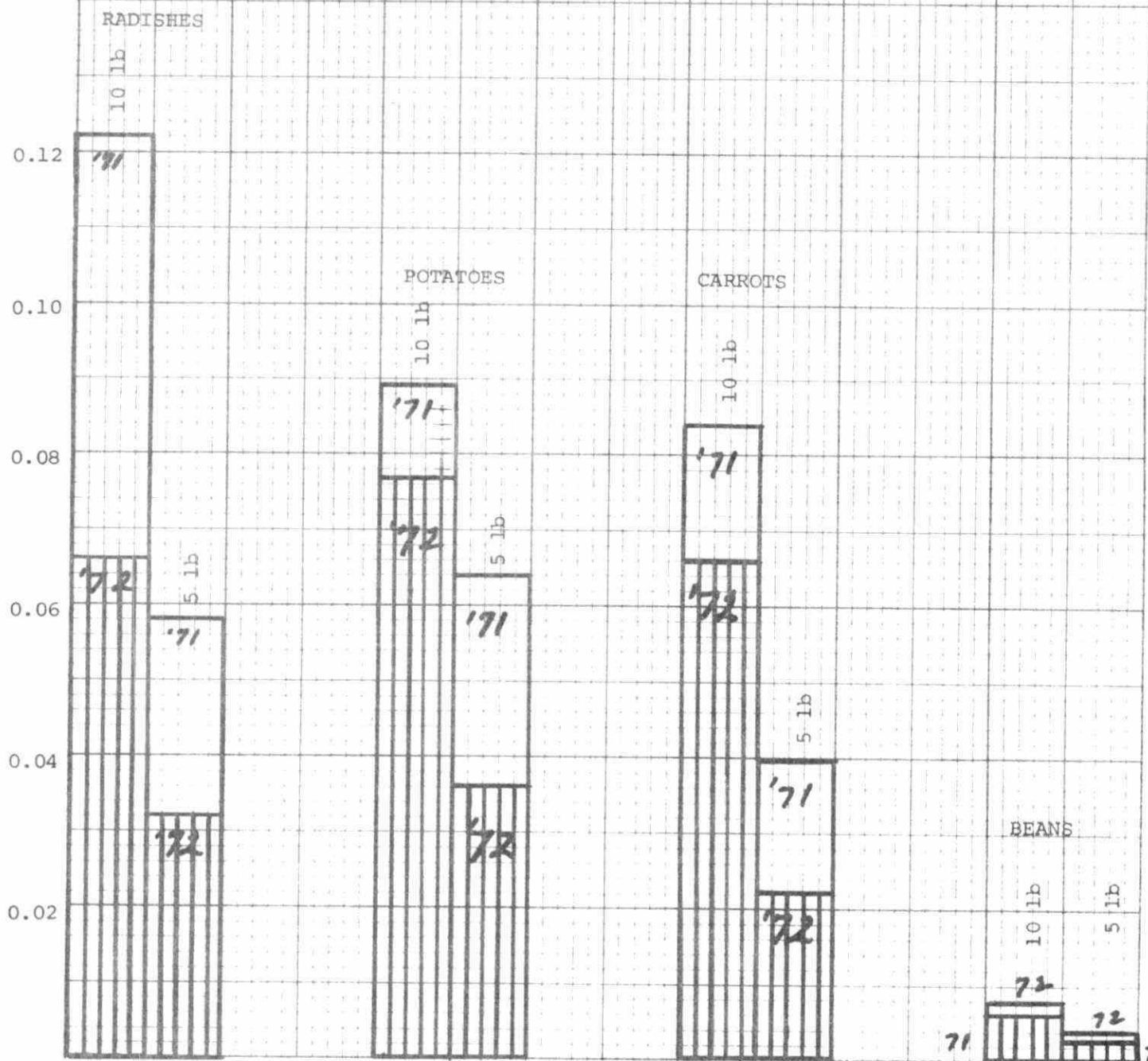


Figure 10. Summary of Crop Residues from HCS-3260 showing the amount of + Chlordane Present in 1971-1972. Langley, B.C.



RESIDUES IN ALFALFA FOLLOWING
SOIL TREATMENT WITH HIGH PURITY CHLORDANE
(Velsicol HCS-3260)

by

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INTRODUCTION

Oxychlordanes, 1,2-dichlorochlordene epoxide (Fig. 1, III), is a recently described metabolite of chlordanes (Fig. 1, I) occurring in some animals fed on chlordanes or chlordanes-treated forage (10). It has been found as a contaminant in milk and cheese from cows fed on alfalfa previously treated with technical chlordanes (3, 4, 9). All reports have indicated that oxychlordanes is formed only in animals and does not appear as a terminal residue in soil or crops (10). According to Street and Blau (11), α - and γ -chlordanes give rise to oxychlordanes in animals via the intermediate 1,2-dichlorochlordene (Fig. 1, II).

Photo-cis-chlordanes (Fig. 2, V) is a photoisomer of α -chlordanes (Fig. 2, IV) similar in structure to photodieldrin. Sunlight or short wave U-V light produces a "half-cage" compound through hydrogen migration and carbon-carbon bond formation (1). In contrast, γ -(trans)-chlordanes does not photolyze to a half-cage structure because of steric hindrances of the chlorine atoms on the cyclopentane ring.

We report the occurrence of 1,2-dichlorochlordene, oxychlordanes, and photo-cis-chlordanes as well as the parent chlordanes compounds in alfalfa after treatment of the soil with Velsicol HCS-3260.

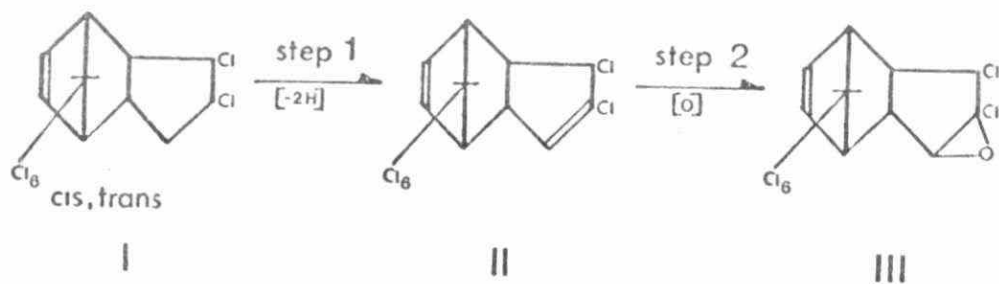


Figure 1. Metabolism of chlordane (I) to 1,2-dichlorochlordene (II) and oxychlordane (III).

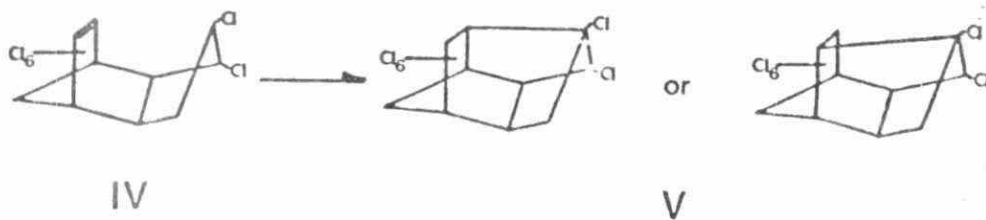


Figure 2. cis-chlordane (IV) and its photoisomers (V) (from Benson et al. (1)).

MATERIALS AND METHODS

Insecticide and Standards

Soils were treated with high purity chlordane, recently developed by the Velsicol Chemical Corp., identified as HCS-3260. Its active ingredients consist of 95% or more of α - (=cis-) and γ - (=trans-) chlordane (12). The ratio of cis-/trans- is appr. 3/1. Analytical reference standards for the α - and γ - isomers, oxychlordane, and 1,2-dichlorochlordene were kindly supplied by the Velsicol Chemical Corp., photo-cis-chlordane by the Canada Department of Agriculture, Analytical Services Section, Ottawa.

Treatment

The alfalfa (Lahontan variety) was grown at the Canada Department of Agriculture Research Station at Summerland, British Columbia. Six plots (20 x 20 ft. with 20 ft. buffer spaces) were staked and the alfalfa within each plot was cut to ground level with a lawn mower and raked clean. Appropriate amounts of HCS-3260 EC were diluted with water and applied to the soil with a HAWS 2-gallon watering can in June, 1971. Four gallons of emulsion were used to cover each plot in one direction; a further 4 gallons were then applied in the opposite direction. The treatment rates were 5 or 10 lbs a.i./acre, in two replicates. Two plots were left untreated as controls. All the plots were thoroughly watered after treatment.

Sampling

Alfalfa was sampled 2 months after soil treatment and cut to about 4 inches 3 months after treatment. The new growth was sampled at 4 months (i.e., 1 month after cutting) and again 1 year after treatment. The samples were washed under running water, dried, and frozen at -20°C until extraction.

Extraction and Cleanup

Subsamples from each plot were macerated and 100 g duplicate aliquots extracted in 400 ml of hexane:acetone 1:1 (v/v) for 30 minutes in a Lourdes homogenizer. After 20 minutes, 25 g of anhydrous Na_2SO_4 were added. The extracts were decanted through glass wool into separatory funnels and the acetone was removed by washing three times with 200 ml 2% aqueous Na_2SO_4 . Twenty ml of the crude hexane extract were concentrated to 2 ml and cleaned on 6 g 100-200 mesh Florisil. Forty ml of hexane followed by 40 ml of benzene:hexane 5:1 (v/v) were used to elute the compounds from the columns. The eluates were taken to dryness in a flash evaporator and the residues taken up with 5 ml hexane.

GLC Analysis

GLC analysis was in a Microtek MT-220 equipped with dual ^{63}Ni electron capture detectors. Three glass columns were used (183 cm x 0.64 cm O.D.), packed with mixtures of: (I) 2% OV 1 + 6% OV 210; (II) 2% SE 30 + 6% QF 1; and (III) 1.50% OV 17 + 1.95% OV 210 on Chromosorb W "H.P.", 80/100 mesh. N_2 was used as carrier gas at 80 ml/min. Common flow to one detector was accomplished by means of a combiner for columns (II) and (III). Purge gas on the EC detector for column (I) was 20 ml/min. There was no purge gas on the EC detector for columns (II) and (III). Temperatures were: injector 220°C , oven 190°C , detectors 300°C . Standard curves were prepared before and after each analysis.

Isolation and Confirmation of Oxychlordanes

Column Chromatography: Analyses of the extracts by GLC indicated the presence of α -, γ -, oxy-, and photo-cis-chlordane. On 6 g Florisil, using 0.2 μg of analytical reference standards of the above compounds, α -, γ -, and photo-cis-chlordane were separated from oxychlordanes by eluting with 60 ml of pet. ether:hexane 2:3 (v/v). Oxychlordanes were then eluted using 40 ml

of benzene:hexane 5:1 as described. For the field samples, 20 ml of alfalfa extract were concentrated, and oxychlordanes were separated from the other metabolites in the same way.

Chemical Derivatization: Reductive dechlorination using aqueous chromous chloride (CrCl_2) solution as described by Cochrane and Chau (6) was performed on 0.8 μg analytical reference oxychlordanes and on approximately 0.3 μg oxychlordanes isolated from the alfalfa extract by column chromatography.

Gas Chromatography: In addition to the GLC columns already described, a more polar 7% OV 225 liquid phase on 60/80 mesh Gas Chrom Q was also used for confirmation. GLC conditions for this column were as described.

p-Value: For hexane-acetonitrile partitioning of both cleaned-up alfalfa extracts and analytical reference oxychlordanes in hexane, we used the method described by Beroza *et al.* (2).

Recovery Tests

Macerated alfalfa tissue from the control plots was fortified with known amounts of analytical standards of α -, γ -, oxy-, and photo-cis-chlordane. Recoveries from duplicate fortified samples were between 94 and 109%.

RESULTS AND DISCUSSION

Figures 3 and 4 show the chlordane residues in alfalfa at the two application rates and three sampling intervals. The initial growth of alfalfa, from 24 to 30 inches high, was sampled 2 months after treatment. It had somewhat higher residues than the new growth sampled 4 months after treatment. Samples taken 1 year after treatment had a marked reduction in residues.

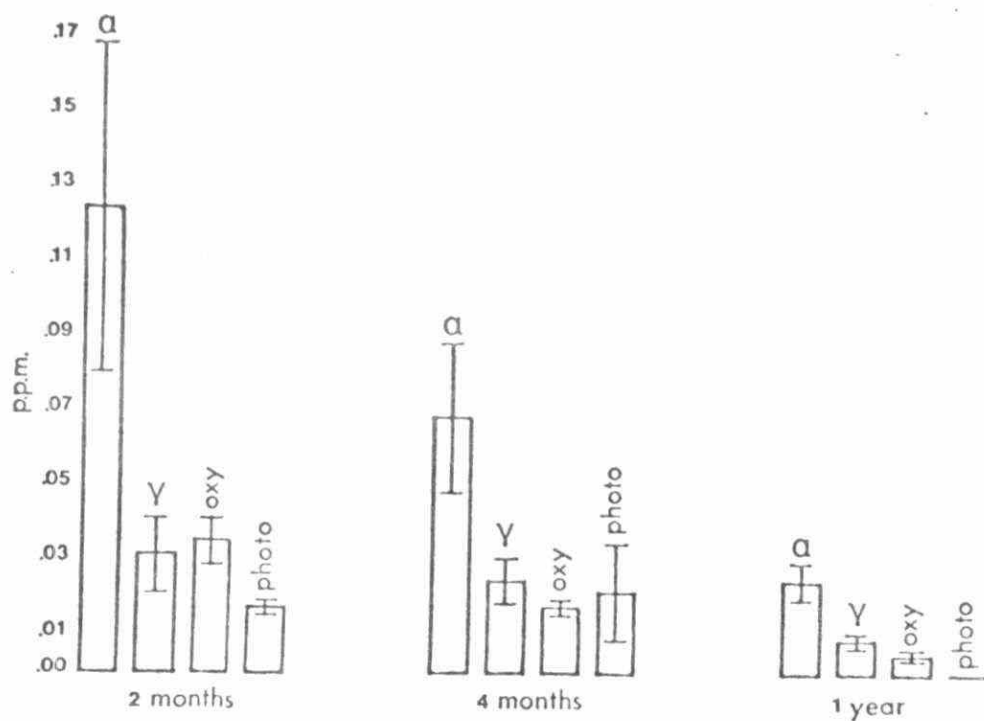


Figure 3. Chlordane residues in alfalfa at intervals following soil treatment of 5 lbs a.i./acre (mean and S.D. in p.p.m. of fresh weight).

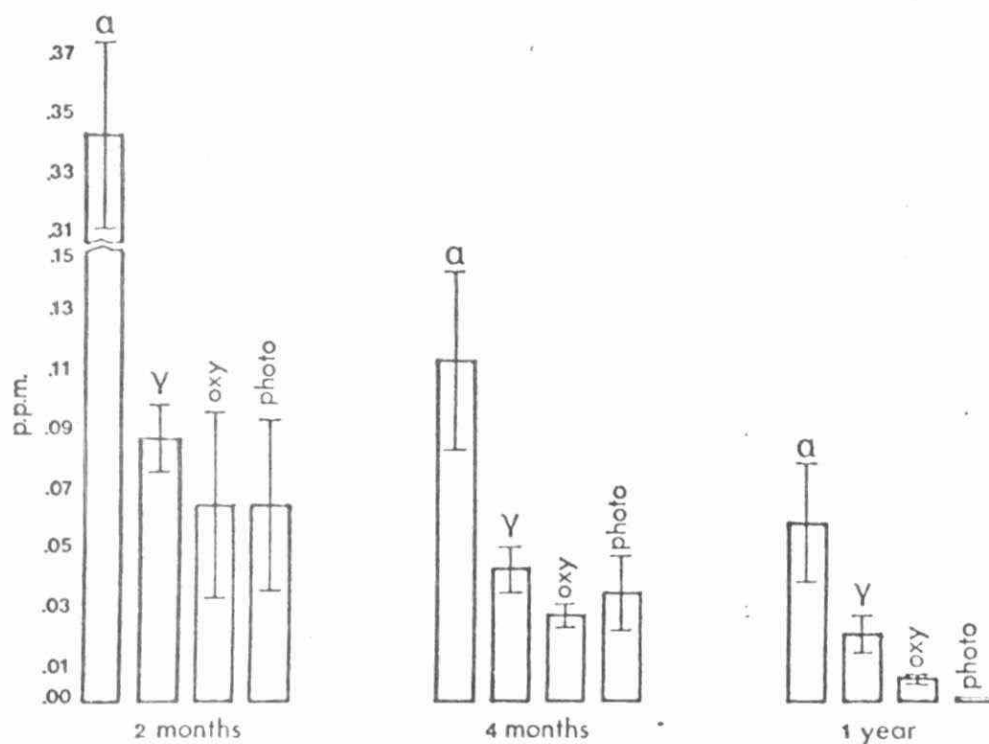


Figure 4. Chlordane residues in alfalfa at intervals following soil treatment of 10 lbs a.i./acre (mean and S.D. in p.p.m. of fresh weight).

In contrast, the concentrations of α - and γ -chlordane in the soil did not decline; similar amounts were detected after 4 months and 1 year (13).

Oxychlordane comprised from 9 to 13% of the total residues (α - + γ - + oxy- + photo-cis-chlordane) in the alfalfa from the plots treated at 10 lbs/acre, and 13 to 17% in that from the plots treated at 5 lbs/acre. It appears to be endogenous since it could not be detected in the soils. The intermediary compound, 1,2-dichlorochlordene, was detected at 0.011 ppm in the alfalfa from the 10-lb plots, and in trace quantities in the other.

Since these results seem to be the first indication that oxychlordane can appear as a residue in plants, further confirmation of identity was very important, especially in view of previous misidentification of oxychlordane for heptachlor epoxide (5). Our columns (I) and (II) gave good separation of, and sensitivity to, these compounds. The separation of α -, γ -, and oxychlordane and heptachlor epoxide on our column (III) has recently been described (8). The more polar OV 225 column also clearly separated oxychlordane from heptachlor epoxide but did not resolve α - and γ -chlordane. GLC analysis of alfalfa samples from the control plots showed no peaks interfering with any of the above-mentioned compounds. p-values for standard and extracted oxychlordane were 0.44 and 0.45 respectively. Standard and extracted oxychlordanes were monodechlorinated with CrCl_2 by attack on the gem-dichloro group present in the hexachlorocyclopentene moiety to yield the sym- and anti-heptachloro-derivatives (7), and then confirmed by GLC.

Photo-cis-chlordane^{1/} accounted for 9 to 16% of the total residues in the alfalfa from both 5- and 10-lb plots. It was also found in the soil (max. 0.15 ppm) for four months following the treatment. The occurrence

^{1/}Also identified by the Analytical Services Section, Canada Dept. of Agriculture, Ottawa.

of this photo-isomer was probably due to a combination of the method of application of HCS-3260, its composition, and the continental, semi-desert climate of the area at that time (Table 1).

TABLE 1

Climatic Conditions for Sampling Period
June to October 1971.*

	Bright sunlight (hours)	Precipitation (inches)	Average air temp. (°F)
June	233.9	2.06	59.5
July	357.9	0.47	69.8
Aug.	351.8	1.19	74.0
Sept.	182.5	0.76	56.6
Oct.	156.1	0.54	46.3

*Compiled from Chapman, F. M. 1972. "Weather Observations for 1971", Canada Dept. of Agriculture, Research Station, Summerland, B. C.

SUMMARY

Residues of chlordane were determined in alfalfa up to one year after application of Velsicol HCS-3260 to soil at 5 and 10 lbs active ingredient per acre. The major residues were cis-, trans-, photo-cis-, and oxy-chlordane. The latter two accounted for a maximum of 16% and 17%, respectively, during the first four months after treatment. The identity of oxychlordane was confirmed by four different GLC columns, chemical derivatization, p-value determination, and column chromatography.

ACKNOWLEDGMENTS

The authors are grateful to the Velsicol Chemical Corp. for financial support, the insecticide, and standards used in this project. We are also thankful to Dr. W. P. Cochrane, Canada Dept. of Agriculture, for identifying and kindly supplying a sample of photo-cis-chlordane. We thank the Canada Dept. of Agriculture Research Station at Summerland, B. C. for the experimental plots and their maintenance.

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YOUR FILE NO
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OUR FILE NO
NOTRE RÉF. N°

Mr. Douglas Wilson,
Pesticides Advisory Committee,
Fifth Floor, Mowat Block,
Queen's Park,
Toronto, Ontario,
M7A 1A2

February 21, 1973

Dear Doug:

You may recall a recent release on "Photo-Decomposition Products of Heptachlor" by Dr. Charles Hammer of the United States. Since he made some rather shocking accusations about the toxicity of some of the so-called photo products, I got in touch with Dr. Percy Polen at Velsicol. Percy has replied to my request and I am enclosing his letter and some literature which he sent. I think that it might be useful to include this information as an appendix in the chlordane-heptachlor report in case anyone brings up the question of the so-called photo products.

Best regards.

Yours truly,

R.

C. R. HARRIS,
Head,
Soil Pesticide Section.

CRH:MACS
Encls.

c.c. Dr. F. L. McEwen

VELSICOL CHEMICAL CORPORATION

341 EAST OHIO STREET • CHICAGO, ILLINOIS 60611 • 312 467-5700

February 13, 1973

Dr. C. R. Harris
Research Institute
University Sub Post Office
Canada Department of Agriculture
London 72, Ontario

Dear Ron:

I refer to our phone conversation of about 2 weeks ago regarding the concern of Canadian authorities with respect to a syndicated news release claiming the discovery of photoalteration products from heptachlor which were "22 times more toxic". More information, useful in evaluating that news item, is on hand now.

These are the facts as we understand them. Charles F. Hammer, Associate Professor, Chemistry Department, Georgetown University, recently presented a paper before the Division of Molecular Spectroscopy at a regional meeting of the American Chemical Society. The paper was entitled Photo-Decomposition Products of Heptachlor Epoxide. As the enclosed typescript shows, it was an interpretation of spectral data obtained from two previously characterized photoisomers of heptachlor epoxide. The author, to introduce his report, draws (incorrectly) upon published works of Ivie, et al. (1972) and Lichtenstein, et al. (1970); he presents no new evidence of agricultural or toxicological nature.

Irrespective of the nature of his own research, Hammer appears to have freely interpreted the biological and agronomic work of other investigators in a press interview which was the basis of the syndicated article. (The Chicago Sun-Times version is enclosed.) The item speaks of detection of the photoproducts in potatoes and carrots, even though Hammer did not work on them. Lichtenstein, presumably Hammer's source, reports no such findings in these crops after heptachlor treatment -- unless heptachlor epoxide is supposed to be a photoproduct. Hammer bases his

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Dr. C. R. Harris

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February 13, 1973

relative toxicity ratings on "LD50, mg/kg housefly (applied with sesamex) = 22" (see Table 1 of manuscript), a figure which he derives from Lichtenstein's paper when, in fact, Lichtenstein's report does not support such a conclusion. Most important of all, Hammer, while citing Ivie's work, failed to convey to the reporters Ivie's observation: "No detectable photoproducts were formed [on plant surfaces] in the absence of rotenone as a photosensitizer;...."

If you have any comments, I would be grateful for them.

Sincerely,

VELSICOL CHEMICAL CORPORATION



Percy B. Polen
Principal Regulatory Scientist

PBP/cf

Enclosures: (4)

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Degradation of Aldrin and Heptachlor in Field Soils

During a Ten-Year Period

Translocation into Crops

E. P. Lichtenstein, K. R. Schulz, T. W. Fuhremann, and T. T. Liang

Data are presented relative to the accumulation following treatment and the subsequent decline of aldrin or heptachlor residues in loam soils during a 10-year period (1958-1968). Loam soils treated with aldrin or heptachlor at 25 pounds per five-inch acre over the five-year period of 1958 through 1962 contained in the fall of 1968, 4 to 5% of the applied dosages primarily in the form of dieldrin and heptachlor epoxide. Aldrin treated soils also contained photo-dieldrin, which amounted to 1.5% of the recovered dieldrin. In addition, three unidentified, more polar compounds were detected in these soils, but they were nontoxic to both vinegar flies and houseflies. In addition to gamma chlordane and

nonachlor which were present in the original heptachlor formulation, two toxic metabolites (heptachlor epoxide and alpha-chlordane) and three unidentified, nontoxic compounds were detected, thus indicating the breakdown in soils of heptachlor and related compounds. All crops grown in these soils contained some insecticidal compounds. Potatoes from aldrin treated soils contained dieldrin (0.047 p.p.m.) and photo-dieldrin (0.0006 p.p.m.), while those grown in heptachlor treated soils contained heptachlor (0.002 p.p.m.), heptachlor epoxide (0.054 p.p.m.), gamma-chlordane (0.015 p.p.m.), alpha-chlordane (0.004 p.p.m.), and nonachlor (0.002 p.p.m.).

During the first 10 to 15 years after the introduction of organic synthetic pesticides, long term residual properties were regarded as desirable. It was thought to be a remarkable feature when it was found that an insecticide like DDT could be applied to mud huts and result in mosquito control over an extended period of time. In fact, it was regarded as desirable to make insecticide residues "last longer and look better" through the addition of "polychlorinated polyphenyls for improving lindane residues" (Hornstein and Sullivan, 1953). This attitude has changed considerably when it was found that some insecticidal residues are persistent and widely distributed and are found in materials where their presence is undesirable. The synthetic organochlorine insecticides degrade in soil, although the rate of this degradation is different for each compound, depending on the nature of the chemical itself and on a variety of environmental factors. Some of these chemicals are more persistent, or less degradable, while others are less persistent and more susceptible to the effects of biological, chemical, and physical factors.

In this study, long term field experiments are described in which the insecticides aldrin or heptachlor were applied to agricultural loam soils. The fate of these insecticides in the soil and their translocation into crops during the ten-year period of 1958-68 are discussed.

PROCEDURE

Soil Treatments at Abnormally High Dosages and Soil Sampling. In May 1958, duplicate 30- X 40-foot Carrington silt loam plots near Madison, Wis., were treated with emulsions of aldrin and heptachlor at 5 or 25 pounds per acre (Lichtenstein, 1960). The soils were then rototilled to a depth of 4 to 5 inches.

Those soils treated at 5 pounds per 5-inch acre were re-treated at the same rate each May from 1959 through 1962.

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At the end of the five years, all plots had been treated in either one or five yearly applications with a total of 25 pounds of insecticide per 5-inch acre (5 X 5 or 25 pounds). These abnormally high treatment rates were chosen, because at the time of the first insecticidal application (1958) colorimetric methods had to be used for analyses. It was also felt that for the reliable detection of potential metabolites higher insecticidal application rates would be desirable.

For soil residue studies, it was intended to determine if and to what extent the insecticides would accumulate in the soil following yearly applications of 5 pounds per 5-inch acre and how fast they would disappear in a subsequent five-year period during which no further insecticidal application was made. These data were compared with data from soils which had received the total 25-pound dosage in one massive application. Insecticide translocation into crops grown in these soils, and of the metabolism of insecticides in soils and crops were also investigated.

Six-inch soil samples were collected as described (Lichtenstein, 1960) immediately after treatment in 1958 and in October of each year. A final soil sample was collected in October of 1968.

Crop Growth and Crop Sampling. During the years 1958-1962 various crops were grown on the insecticide treated plots as previously described (Lichtenstein and Schulz, 1965). From 1963 through 1967, however, only carrots (Red Cored Chantenay) and potatoes (Russet Sebago) were grown as indicator crops, while during the 11th growing season (1968) radishes (Early Scarlet Globe), beets (Detroit Dark Red), and cucumbers (Straight Eight) were grown in addition to carrots and potatoes for translocation studies. Crop sampling and processing was performed according to a previously described procedure (Lichtenstein and Schulz, 1965).

Analytical Methods. Various analytical methods were employed during the 10-year (11 growing seasons) duration of this experiment. Soil and crop samples obtained through 1961 were extracted, cleaned up, and analyzed colorimetrically as described (Lichtenstein, 1960). Samples that were obtained

in 1962 were analyzed by both colorimetric and gas-liquid chromatographic (GLC) methods (Lichtenstein *et al.*, 1964). Data secured by those two methods showed good agreement. Soil and crop samples obtained during the remainder of the period (1963 through 1968) were extracted and analyzed by GLC as described by Lichtenstein *et al.* (1967). In addition, soils and crops grown in 1968 on heptachlor treated plots were analyzed for γ -chlordane. All crops that were grown in 1968 were also tested for the presence of toxic substances by exposing vinegar flies (*Drosophila melanogaster* Meig) to the dry residue obtained from crop extracts (Edwards *et al.*, 1957).

Metabolite Studies in 1968. CHEMICALS USED. Soil and crop extracts from aldrin treated soils were compared with analytical grade aldrin, dieldrin, photo-aldrin or the photo isomer of aldrin (1,1,2,3,3a,7a-hexachloro-2,3,3a,3b,4,6a,7,7a-octahydro-2,4,7-metheno-1H-cyclopenta(a)-pentalene), photo-dieldrin or the photo isomer of dieldrin (1,1,2,3,3a,7a-hexachloro-5,6-epoxydecahydro-2,4,7-metheno-1H-cyclopenta(a)-pentalene), "aldrin-OH" (6,7-trans-dihydroxy-dihydro-aldrin or trans-aldrin diol) a metabolite obtained by Korte and Arent (1965) from rabbit urine after oral administration of dieldrin, and dicarboxyl aldrin (1,2,3,4,10,10-hexachloro-6,7-dicarboxyl-1,4-endo-5,8-exodimethano-1,4,4a-5,6,7,8,8a-octahydronaphthalene.) These chemicals were obtained through the courtesy of the Shell Chemical Company.

Soil and crop extracts from heptachlor treated soils were compared with analytical grade heptachlor, heptachlor epoxide, chlordene (4,5,6,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-4,7-endomethanoindene), chlordane (α and γ isomer) (2,3,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-endomethanoindene), nonachlor (delta-trichloro-chlordene) (1,2,3,4,5,6,7,8,8-nonachloro-2,3,3a,4,7,7a-hexahydro-4,7-endomethanoindene). All these compounds were obtained through the courtesy of the Velsicol Chemical Corporation.

To determine the toxicity of these various compounds, 50 vinegar flies were exposed in each of two bioassay jars to the dry residue of 20 μ g of each of these chemicals. Approximate 50% mortalities were obtained with aldrin or dieldrin in 2.5 hours, photo-aldrin in 1.5 hours, photo-dieldrin in 2.5 hours, heptachlor or heptachlor epoxide in 1 hour, α -chlordane or γ -chlordane in 3 hours, nonachlor in 16 hours, and chlordene in 24 hours. No toxicity effects were obtained during a 48-hour exposure period with "aldrin-OH," dicarboxyl aldrin, and "1-OH chlordene" (1-hydroxy-4,5,6,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-4,7-endomethanoindene).

SOILS, QUALITATIVE MEASUREMENTS. Soil samples collected in October of 1968 were examined for the presence of metabolites that could have been produced in addition to dieldrin or heptachlor epoxide. Tests were also conducted to determine the presence of compounds that could have remained in the soil after application as impurities in the original insecticide formulation. Soils were extracted in a homogenizer with redistilled acetonitrile (2 ml per gram of wet soil), followed by concentration of the extract to 0.2 ml at 25° C. in a flash evaporator. One fifth of this concentrate, usually representing 40 grams of wet soil, was then analyzed by thin-layer chromatography (TLC). The concentrate was spotted on aluminum oxide G coated glass plates (5 × 20 cm), 2.5 cm above the lower edge. Chromatograms from aldrin treated soils were developed with isooctane-pyridine (7 to 3) or with isooctane-diethyl ether (7 to 3), while those from heptachlor treated soils were developed with isooctane or with cyclohexane-ethylacetate (7 to 3). The separated compounds were visualized by spraying with reagents as described by

Mitchell (1957) and subsequent exposure to ultraviolet light for 10 minutes.

After the first thin-layer chromatograms had been developed, the same spotting procedure was repeated except that two portions of the concentrate were spotted side by side. After development of the chromatogram, one half of the plate was covered with aluminum foil and the other half sprayed and visualized as described. The foil-covered unsprayed portions of the aluminum oxide G layers corresponding to each of the different spots observed on the sprayed side of the plate were then scraped off and extracted with a 1 to 12 mixture of acetonitrile and acetone. Aliquots of these extracts (approximately 0.5 ml) were then evaporated to dryness, the residue was redissolved in 0.5 ml of hexane followed by analyses by GLC.

The same acetonitrile-acetone extracts were also used for toxicity tests with vinegar flies (Edwards *et al.*, 1957) and houseflies (*Musca domestica*, C.S.M.A., 1948 strain). With vinegar flies, aliquots of the extracts representing 36 grams of soil were pipetted into bioassay jars and the solvents were evaporated in a fume hood. To test toxic effects resulting from contact with the residue or by vapors emanating from this residue, 50 flies were introduced into each of two bioassay jars. Mortality counts were performed at intervals during a 45-hour exposure period. When houseflies were used, aliquots of the acetonitrile-acetone extracts were evaporated to dryness, then redissolved in 0.5 ml of acetone. One microliter of this acetone solution representing 100 mg or 300 mg of soil was then applied topically to the ventral portion of the abdomen of female houseflies. Mortality counts were performed 45 hours later.

For the qualitative determination of potential water soluble metabolites, soil samples were extracted with acetonitrile (2 ml per gram of soil). The resulting solution was then concentrated at 45° C. in a flash evaporator to approximately 10 ml, to which 100 ml of water was added. This mixture was then re-extracted with three 50-ml portions of hexane. The water-acetonitrile phase was evaporated to dryness at 45° C., the residue was redissolved in small amounts of acetone and spotted on an aluminum oxide G coated glass plate. The chromatogram was then developed with isooctane-pyridine (7 to 3) and sprayed as described. Aliquots of the hexane phase were handled in the same way. Finally, isolates were prepared from these plates as described and also analyzed by GLC.

SOILS, QUANTITATIVE MEASUREMENTS. To measure actual amounts of the various metabolites, soil samples were extracted with acetonitrile (2 ml per gram of soil), followed by diluting the extract with water (5 ml per gram of soil) and partitioning of the insecticidal residues into hexane. After the hexane had been dried over anhydrous sodium sulfate, it was adjusted to volume and appropriate aliquots were used for analyses by GLC. Added amounts of aldrin, dieldrin, heptachlor, or heptachlor epoxide were recovered to an extent of 90-95%.

POTATOES, QUALITATIVE AND QUANTITATIVE MEASUREMENTS. Because of minimal analytical interference, potatoes were used as the primary test plant to determine the presence of aldrin or heptachlor metabolites in a crop. Potatoes and some samples of carrots were extracted with acetonitrile as described for soils (quantitative measurements) and partitioned into hexane. The hexane fraction was then concentrated and cleaned up by passing it through a 10-gram Florisil (PR grade, 60- to 80-mesh) column using 150 ml of 15% diethylether in hexane as the eluting solvents. This

Table I. Recoveries of Aldrin (A) and Dieldrin (D) Residues from Soils and Crops Grown in 1968 on Aldrin-Treated Field Plots

	Aldrin Applied to Soil, Lb/5-Inch Acre			25 ^b		
	5 × 5 ^a	Recovered in Fall of 1968, P.P.M. ^c		25 ^b		
	A + D	%D ^d	CR %S ^e	A + D	%D	CR %S
Soil	0.860	99	...	0.690	98	...
Carrots	0.129	100	15.0	0.176	100	25.4
Potatoes	0.044	100	5.1	0.046	100	6.6
Beets	0.048	100	5.6	0.053	100	7.7
Radish	0.085	100	10.0	0.078	100	11.4
Cucumber	0.102	100	12.8	0.122	100	17.8

^a Aldrin applied at 5 lb/5-inch acre in May of each year (1958 through 1962). Total application: 25 lb/acre (15.6 ppm) over 5-year period.

^b Aldrin applied at 25 lb/5-inch acre (15.6 ppm) in May 1958 only.

^c Results are averages of duplicate field plots.

^d Dieldrin in per cent of total residue recovered (A + D).

^e CR %S = crop residue in % of soil residues.

cleaned up extract was concentrated to volume and analyzed by GLC and TLC.

Extracts from potatoes that were grown on aldrin treated soils were qualitatively analyzed by TLC using aluminum oxide G as the coating and isoctane-pyridine (7 to 3) as the moving solvent system. Areas corresponding to R_f values obtained with dieldrin and photo-dieldrin were scraped off the plate and tested by GLC as described. For quantitative residue determinations aliquots of the cleaned up diethylether-hexane extract were also analyzed directly by GLC.

Extracts from potatoes that were grown on heptachlor treated soil were analyzed qualitatively and quantitatively as described. However, isoctane was used as the moving solvent for TLC and nonsprayed areas that corresponded to all the visualized spots (Figure 4) obtained from potato extracts were scraped off the plate and tested by GLC and *Drosophila* bioassay.

RESULTS AND DISCUSSION

Insecticide residue levels in soils treated with aldrin or heptachlor at 5 pounds per acre per year from 1958 through 1962 are presented in Figure 1 for the 10-year period 1958–68. Data for soils, carrots, and potatoes represent the total of aldrin plus dieldrin or of heptachlor plus its epoxide. Dieldrin was produced within the soil from aldrin and amounted to 50 and 90% of the total aldrin plus dieldrin recovered in 1959 and 1963, respectively. Heptachlor epoxide was produced from heptachlor at a slower rate and reached the 50% level in the fall of 1964 and the 90% level in the fall of 1968.

During the period of annual insecticide soil treatments residue levels increased steadily through 1962, when their concentrations in soil amounted to 19% of the totally applied insecticide dosage of 25 (5 × 5) pounds per acre. In subsequent years, when no further soil treatments were performed, residue levels declined at a relatively slow rate. In fall of 1968, 5.3% (aldrin plus dieldrin) and 4.6% (heptachlor plus heptachlor epoxide) of the applied aldrin or heptachlor were detected in these soils.

Insecticide residues were also absorbed by crops grown in these soils, with carrots absorbing the largest amounts (Figure 1). Although residue levels in soils increased up to 1962, the residue concentration in both carrots and potatoes reached its peak during the 1960 growing season. During that year, the concentration of aldrin plus dieldrin in carrots was 1.08 ppm and of heptachlor plus heptachlor epoxide 1.90 ppm. Residue levels in potatoes never exceeded 0.30 to 0.32 ppm (1960–62) of aldrin plus dieldrin or 0.54 to 0.51 (1960–62) ppm of heptachlor plus its epoxide. Apparently a threshold had been reached beyond which the content of insecticidal residues remained constant in these two crops. If more residues were absorbed, they could have been metabolized by the plant tissue into compounds that were not detected at that time. When insecticide residue levels in soils started to decline (1963), both carrots and potatoes also contained proportionally smaller amounts of residue.

For analyses of samples obtained in 1968, both soils and some of the crops were extracted by two procedures as described. Results obtained after samples had been extracted with a 1 to 1 mixture of hexane and acetone and analyzed by GLC for aldrin and dieldrin are summarized in Table I.

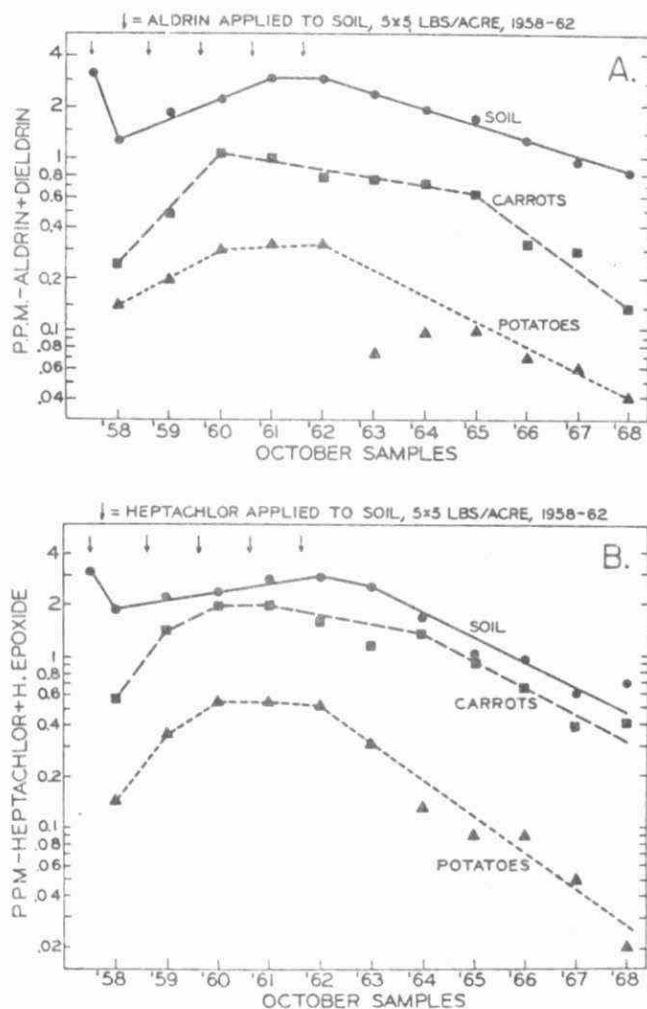


Figure 1. Aldrin plus dieldrin and heptachlor plus heptachlor epoxide residues in soils and their translocation into crops, after 5 yearly soil applications (1958–62) of aldrin or heptachlor at 5 lbs/5-inch acre

Table II. Recoveries of Heptachlor (H), Heptachlor Epoxide (HO), and γ -Chlordane (γ -Ch) Residues from Soils and Crops Grown in 1968 on Heptachlor-Treated Field Plots

Heptachlor ^a Applied to Soil, Lb/5-Inch Acre										
5 × 5 ^b						25 ^c				
Recovered in Fall of 1968, P.P.M. ^d										
	H + HO	%HO ^e	CR% ^f S ^f	γ-CH	CR% ^e S	H + HO	%HO	CR% ^e S	γ-CH	CR% ^e S
Soil	0.701	89	...	0.817	...	0.719	93	...	0.925	...
Carrots	0.413	92	58.0	0.136	16.6	0.223	98	31.2	0.075	8.1
Potatoes	0.070	98	9.5	0.016	2.0	0.064	100	8.8	0.023	2.5
Beets	0.057	100	8.1	0.015	2.0	0.052	100	7.2	0.013	1.4
Radish	0.139	100	19.8	0.027	3.3	0.130	100	18.1	0.031	3.3
Cucumber	0.085	95	12.0	0.022	2.7	0.068	100	9.4	0.024	2.6

^a Formulation contained in addition to one pound of actual heptachlor 0.25–0.3 pounds of γ -chlordane.

^b Heptachlor applied at 5 lb/5-inch acre in May of each year (1958 through 1962). Total application: 25 lb/acre (15.6 ppm) over 5-year period.

^c Heptachlor applied at 25 lb/5-inch acre (15.6 ppm) in May 1958 only.

^d Results are averages of duplicate field plots.

^e Heptachlor epoxide in per cent of total residues recovered (H + HO).

^f CR%^eS = crop residue in % of soil residue.

Soils that had been treated with aldrin at five yearly dosages of 5 pounds per acre contained in the fall of 1962 more aldrin plus dieldrin residues than those that had been treated with one 25-pound-per-acre dose in 1958 (Lichtenstein and Schulz, 1965). By 1968, these differences had nearly disappeared: 5.3 and 4.4% of the total aldrin applied were recovered from these soils in the form of aldrin and dieldrin. Residues in crops were all in the form of dieldrin, but varied in their concentration according to the particular crop. The highest dieldrin concentration was found in carrots, followed by cucumbers, radishes, beets, and potatoes.

Table II summarizes data from analyses of samples obtained in 1968 from heptachlor treated soils and from crops grown therein. The commercial formulations of heptachlor used contained in addition to 1 pound of actual heptachlor 0.25 to 0.3 pound of γ -chlordane and 0.04 to 0.1 pound of "other compounds" which are largely in the form of nonachlor (Velsicol Chemical Corp., 1967). During the five-year soil treatment, a total of 25 pounds of actual heptachlor had been applied which also resulted in an application of 6.25 to 7.5 pounds of γ -chlordane and 1.0 to 2.5 pounds of nonachlor. Since chlordane is more persistent than heptachlor (Lichtenstein and Polivka, 1959), more γ -chlordane than heptachlor and heptachlor epoxide was present in the soil after 10 years.

In the fall of 1968 heptachlor plus heptachlor epoxide concentrations in soils amounted to 4.5% of the total heptachlor applied (Table II). They were similar to concentrations of aldrin plus dieldrin in aldrin treated soils. In fall of 1968, 18.5 and 21% of the total γ -chlordane applied was still in the soil. A total of 32 pounds of heptachlor and γ -chlordane had been applied to these soils and close to 8% of that combined total was recovered from the soil in the form of heptachlor, heptachlor epoxide, and γ -chlordane.

Crops grown in 1968 on these soils primarily contained heptachlor epoxide and γ -chlordane (Table II). Although more γ -chlordane than heptachlor epoxide was present in the soil, the amounts of γ -chlordane in crops were only one fourth of the heptachlor epoxide concentration. Proportionally more heptachlor epoxide than γ -chlordane had been absorbed by these vegetables.

Bioassay procedures with vinegar flies showed that all crop extracts from both aldrin and heptachlor treated soils caused appreciable insect mortalities during a 48-hour exposure period. No mortalities, though, were observed with extracts from crops that were grown as controls on insecticide free soil.

Metabolite Studies in 1968 of Aldrin-Treated Soils and Crops Grown Therein. SOILS. QUALITATIVE MEASUREMENTS. Photographs of thin-layer chromatograms obtained with extracts of aldrin treated soils are presented in Figure 2. They show the presence of five to seven spots depending on the solvent system used. When eluates from the area corresponding to aldrin (R_f 0.72) were analyzed by GLC, small peaks were obtained with retention times identical to aldrin. In addition to the originally applied insecticide, dieldrin and photo-dieldrin were detected by both TLC and GLC. The presence of photo-aldrin could not be confirmed by GLC but its area contained traces of dieldrin with the isooctane-pyridine system. Eluates from areas corresponding to "aldin-OH" (*trans*-aldin diol) were silanized (Ludwig and

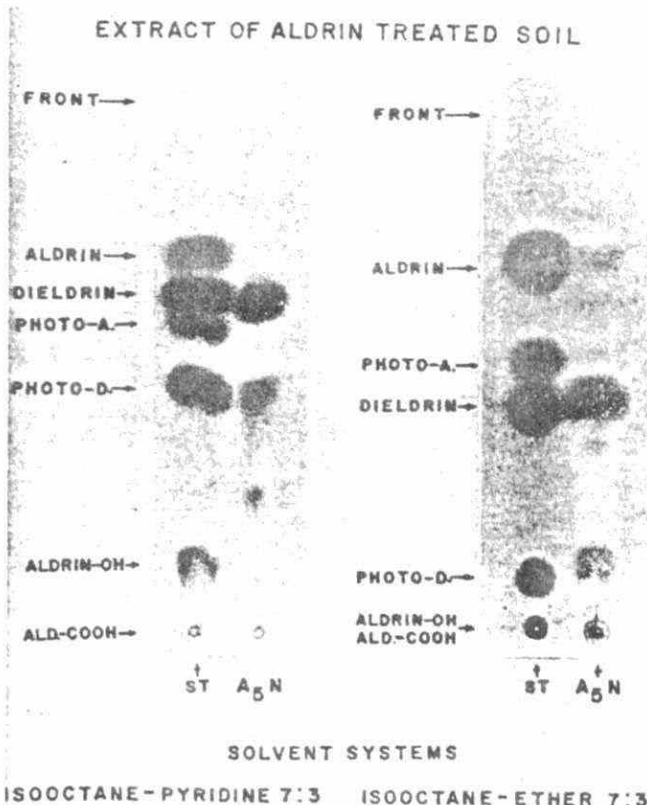


Figure 2. Thin-layer chromatogram of extracts from soil (A.N), treated with aldrin at 5 X 5 lbs/5-inch acre (1958–62) and sampled in 1968

ST = reference compounds

Table III. Toxicity to Vinegar Flies and Houseflies of Aldrin Metabolites Isolated from Soil Extracts by Thin-Layer Chromatography

TLC Solvent System: Isooctane-Pyridine 7:3

R_f	Same as	Per Cent Mortality/45 Hours			
		Drosophila ^a		Musca ^b	
		Contact	Vapor	100 mg	300 mg
0.72	Aldrin ^c	100 ^d	28	100	...
0.63	Dieldrin ^c	100 ^e	16	73	...
0.57	Photo-A ^c	100 ^e	20	20	42
0.46	Photo-D ^c	0	0	0	0
0.26	?	0	0	0	0
0.12	Aldrin-OH	0	0	0	0
0.04	?	0	0	0	0

^a Vinegar flies exposed directly (contact) or to vapors of the dry residue of isolates from thin-layer plates, representing 36 grams of soil each.

^b One μ l of acetone containing the residue from 100 mg or 300 mg of soil isolated from thin-layer plates, was applied topically to the ventral portion of the abdomen of each housefly.

^c Confirmed by GLC.

^d 50% in 6 hours.

^e Area contained dieldrin, as determined by GLC, but no photo-aldrin.

^f 56% in 6 hours.

^g 36% in 6 hours.

Korte, 1965) prior to analyses by GLC but no "aldrin-OH" could be detected. "Aldrin-COOH" (dicarboxyl aldrin) did not move with either solvent system and was not detectable by GLC under the described conditions. Results thus indicated that the major metabolites detected in the soil were dieldrin and photo-dieldrin. With isooctane-pyridine as the solvent system, three unknown compounds were found in ad-

dition to dieldrin and photo-dieldrin. Their R_f values were 0.26, 0.12, and 0.04, thus indicating more polar properties than those of aldrin, dieldrin, or photo-dieldrin.

Table III summarizes the results obtained after vinegar flies or houseflies had been exposed to isolates from the thin-layer plates which had been developed with isooctane-pyridine (7 to 3). Spots containing dieldrin were most toxic to the insects because of the presence of relatively large amounts of this insecticide. The eluate from the area corresponding to photo-aldrin (R_f 0.57) exhibited toxicity, although this compound could not be confirmed by GLC. As mentioned previously, dieldrin was also found in this area, thus accounting for the toxicity. The isolated photo-dieldrin was least toxic because of its much lower concentration in the soil. The three unknown compounds of increasing polarity (R_f 0.26, 0.12, and 0.04) found in aldrin treated soils could not be detected in control soils. They were nontoxic to insects under the described conditions.

To detect potential water soluble metabolites, the water phase obtained from an acetonitrile extract was investigated by TLC and GLC as described. Traces of aldrin and dieldrin were found in the water, plus two peaks whose retention times were 3.0 and 3.4 in relation to the retention time of aldrin. No attempt was made to further characterize these two unknowns.

SOILS, QUANTITATIVE MEASUREMENTS. Samples obtained in 1968 from soil that had been treated with aldrin at five yearly dosages of 5 pounds per acre from 1958-62 were extracted with acetonitrile and contained 0.005 ppm of aldrin, 0.930 ppm of dieldrin, and 0.015 ppm of photo-dieldrin (1.6% of dieldrin). These latter compounds represent the two major toxic metabolites in addition to the three un-

EXTRACT OF HEPTACHLOR TREATED SOIL

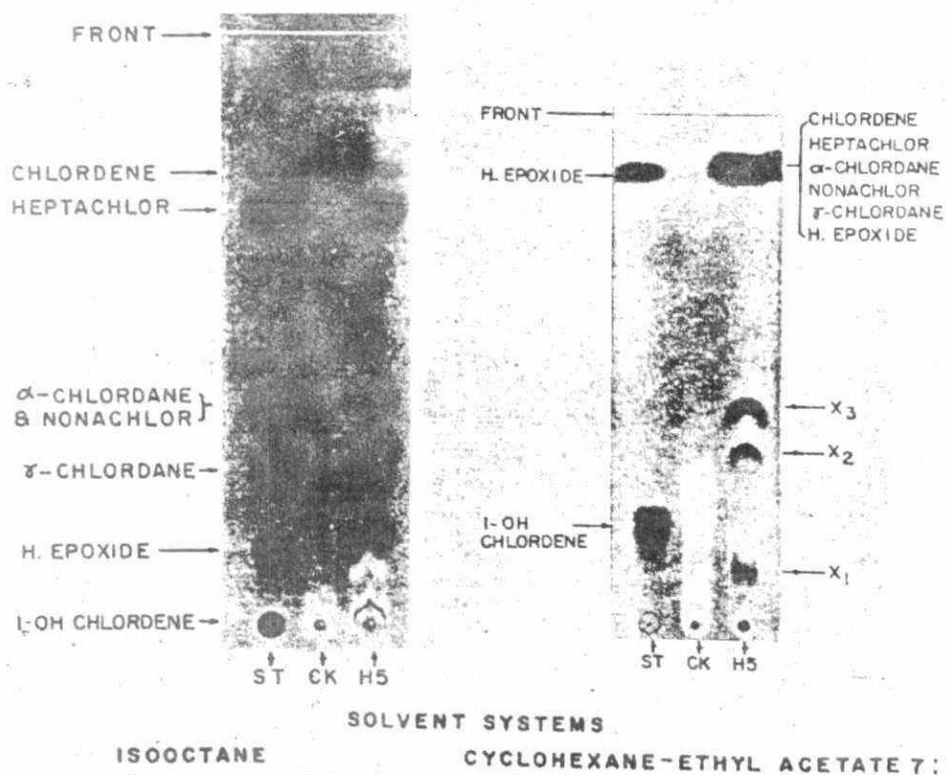


Figure 3. Thin-layer chromatogram of extracts from soil (H₅), treated with a heptachlor formulation at 5 x 5 lbs/5-inch acre (1958-62) and sampled in 1968

ST = reference compounds, CK = extract from insecticide free soil, X₁, X₂, X₃ = unknown compounds

identified and nontoxic compounds which appeared in soil because of the original aldrin application.

POTATOES AND CARROTS, QUALITATIVE AND QUANTITATIVE MEASUREMENTS. Potatoes contained dieldrin (0.047 ppm) and photo-dieldrin (0.0006 ppm) which amounted to 1.3% of the dieldrin concentration. "Aldrin-OH" and "aldrin-COOH" did not pass through the florisil column with 15% diethylether in hexane and could therefore not be detected. Analyses of carrots by TLC was inconclusive because of the presence of interfering substances. Analyses by GLC, though, showed the presence of dieldrin (0.133 ppm) and photo-dieldrin (0.002 ppm), which amounted to 1.5% of the dieldrin concentration.

Metabolite Studies in 1968 of Heptachlor-Treated Soils and Crops Grown Therein. **SOILS, QUALITATIVE MEASUREMENTS.** Results obtained by TLC with extracts from heptachlor treated soils are presented in Figure 3. With isooctane as the solvent, spots were obtained whose R_f values were similar to those secured with reference grade chlordene, heptachlor, α -chlordane plus nonachlor, γ -chlordane and heptachlor epoxide. In addition, three unknown spots (R_f 0.08, 0.48, and 0.60) were observed. With cyclohexane-ethylacetate (7 to 3) as the solvent system 1-OH-chlordene had an R_f value of 0.21 while all the other compounds did not separate, yielding one spot (R_f 0.89) that corresponded to heptachlor epoxide reference compound in Figure 3. Three unidentified spots (X_1 , X_2 , and X_3 at R_f 0.11, 0.34, and 0.43) were visualized with this solvent system although they may not have been identical to those obtained with isooctane as the solvent. To separate α -chlordane from nonachlor, an additional thin layer chromatogram was prepared from heptachlor-treated soil. The chromatogram was also developed with isooctane but the unsprayed area corresponding to α -chlordane plus nonachlor was removed from the plate, extracted with acetonitrile and re-spotted onto a second thin-layer plate. This chromatogram was then developed with 2% diethyl ether in isooctane and resulted in a clear separation of α -chlordane and nonachlor, thus confirming the presence of these two compounds in the soil.

Analyses by GLC of extracts from isooctane developed thin-layer plates confirmed the presence of heptachlor, nonachlor, α -chlordane, γ -chlordane, and heptachlor epoxide (Table IV). These extracts were also toxic when tested with vinegar flies and houseflies. The lower mortalities observed with houseflies probably resulted from the fact that equivalents of only 100 mg of soil were applied per fly. Extracts obtained from the areas of the unknown compounds were nontoxic to the insects. This data, therefore, indicates that after the application of a heptachlor formulation to soil two toxic metabolites (heptachlor epoxide and α -chlordane) and three nontoxic compounds were formed, indicating the breakdown in the soil of heptachlor and related compounds.

TLC of the water-acetonitrile phase, obtained by an acetonitrile extraction of soils as described, revealed the presence of one spot (R_f 0.00) which was not comparable to spots obtained with any of the reference materials used. The hexane phase, though, contained all the other previously described compounds.

SOILS, QUANTITATIVE MEASUREMENTS. Soil samples from plots that had been treated with heptachlor at 5 pounds per acre per year over the five-year period 1958-62 were also extracted with acetonitrile and analyzed quantitatively as described. Concentrations of 0.105 ppm of heptachlor, 0.511 ppm of heptachlor epoxide, 0.769 ppm of γ -chlordane, 0.092 ppm of α -chlordane, and 0.047 ppm of nonachlor were found.

Table IV. Toxicity to Vinegar Flies and Houseflies of Heptachlor Metabolites Isolated from Soil Extracts by Thin-Layer Chromatography

TLC Solvent System: Isooctane

R_f	Similar to	Per cent Mortality/45 Hours		
		Drosophila ^a		Musca ^b
		Contact	Vapors	100 mg
0.77	Chlordene	0	0	7
0.70	Heptachlor ^c	36	22	22
0.61	?	0	0	0
0.48	?	0	0	0
0.39	Nonachlor ^c	85	...	0
	α -Chlordane ^c	94	...	11
0.30	γ -Chlordane ^c	100 ^d	100	11
0.16	H. epoxide ^c	100 ^e	100	69
0.05	1-OH-Chlordene	0	0	0

^a Vinegar flies exposed directly (contact) or to vapors of the dry residue of isolates from thin-layer plates, representing 36 grams of soil each.

^b One μ l of acetone containing the residue from 100 mg of soil isolated from thin-layer plates, was applied topically to the ventral portion of the abdomen of each housefly.

^c Confirmed by GLC.

^d 73% in 6 hours.

^e 100% in 2 hours.

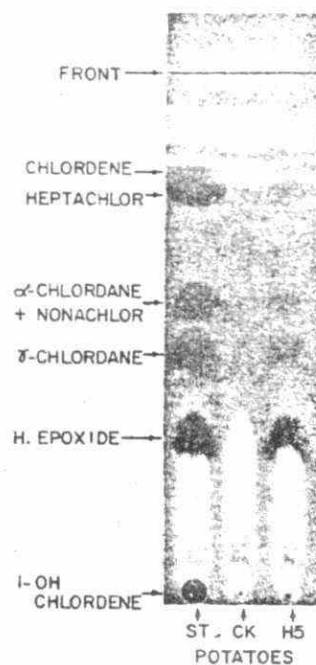


Figure 4. Thin-layer chromatogram of extracts from potatoes grown in heptachlor treated soil (H₅). Solvent: isooctane

ST = reference compounds,
CK = extract from potatoes
grown in insecticide free soil

POTATOES, QUALITATIVE AND QUANTITATIVE MEASUREMENTS. Figure 4 is a photograph of a thin-layer chromatogram obtained with an extract from 72 grams of potato tissue. Accordingly, four spots were found which had R_f values identical with those obtained with reference grade heptachlor, α -chlordane plus nonachlor, γ -chlordane, and heptachlor epoxide. Isolation of these spots and analyses by GLC as described resulted in peaks that were identical with reference

grade heptachlor, γ -chlordane, and heptachlor epoxide. Small peaks, indicating trace amounts of both α -chlordane and nonachlor were also found. These results are qualitatively similar to those obtained with soils, except that the three unknown compounds recovered from soils were not found in potatoes.

To test the biological activity of these compounds, vinegar flies were exposed to isolates from the thin-layer plates. All four spots exhibited some toxicity. After a 48-hour exposure period, mortalities amounted to 8% with isolates from the heptachlor spot, 52% with isolates from the α -chlordane plus nonachlor spot, 64% with isolates from the γ -chlordane spot, and 100% with isolates from the heptachlor epoxide spot. Exposure of flies to isolates corresponding to R_f values obtained with chlordane and "1-OH chlordane" did not result in insect mortality.

Quantitative analyses of potatoes showed the presence of 0.002 ppm of heptachlor, 0.004 ppm of α -chlordane, 0.002 ppm of nonachlor, 0.015 ppm of γ -chlordane, and 0.054 ppm of heptachlor epoxide.

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Novel Photoproducts of Heptachlor Epoxide, *Trans*-Chlordane, and *Trans*-Nonachlor

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The single major photoproduct which has been obtained on exposure of heptachlor epoxide [I, Fig. 1] in acetone solution to ultraviolet light or as thin films to sunlight or a germicidal lamp (1,2) is reported to be the half-cage isomer [IIIA](2), although the isomeric structure [IIIB] also merits consideration (1). Under the same conditions, *cis*-chlordane [IV] yields a photoproduct which is considered to be the half-cage isomer [VA](2) or one of the two isomeric structures [VA or VB](1). In contrast, no half-cage photoisomers have been isolated following exposure of *trans*-chlordane [VI] or *trans*-nonachlor [VIII] to photolytic conditions (1,2,3,4). With these *trans* compounds, the orientation of the chlorine atom on the center carbon of the cyclopentane ring towards the double bond apparently precludes bridging at this position (1).

This paper describes the nature and toxicity of three novel photoisomers obtained when heptachlor epoxide, *trans*-chlordane, and *trans*-nonachlor are exposed to sunlight as deposits on bean foliage, in the presence of rotenone (5), and to ultraviolet light as solutions in acetone. The conversion of heptachlor epoxide to the half-cage isomer [IIIA or IIIB] involves an intermediate [II] which has been isolated and characterized. A new photoisomer of a novel type has also been obtained from each of *trans*-chlordane and *trans*-nonachlor.

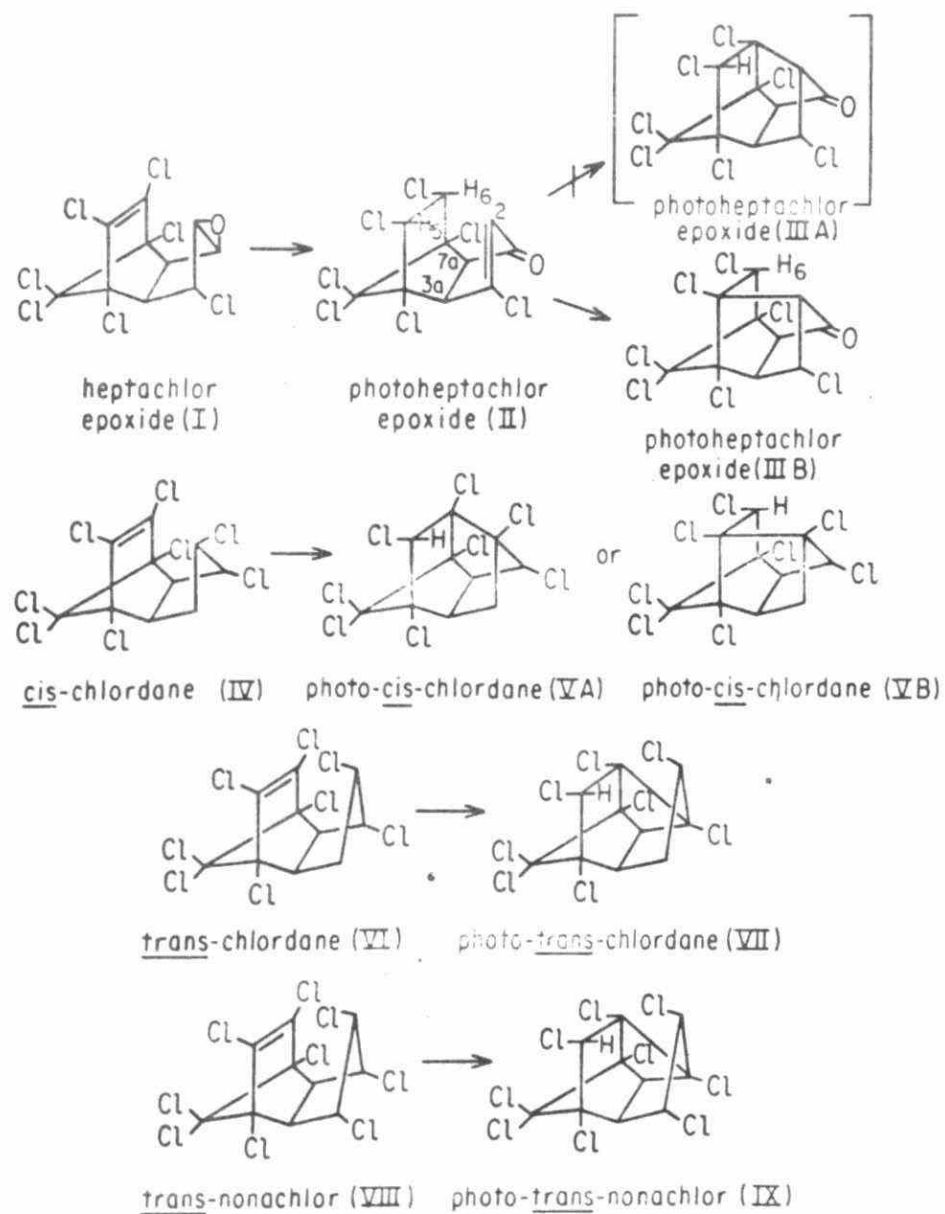
Materials and Methods

Reference chemicals, analytical procedures, and toxicity tests. Reference samples of compounds I, III, IV, V, VI, and VIII were provided by P. R. Delin, Helical Chemical Corp., Chicago, Ill.). The formation of photoproducts was monitored by thin-layer chromatography (TLC) on silica gel F₂₅₄ chromatoplates, using petroleum ether-chloroform mixture (8:1) for heptachlor epoxide and its photoproducts, petroleum ether for *cis*-chlordane and photo-*cis*-chlordane, hexane-ether mixture (4:1) for *trans*-

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Fig. 1. Structures of heptachlor epoxide [I], cis-chlordanes [IV], trans-chlordanes [VI], trans-nonachlor [VIII] and some of their possible photoproducts



chlordanes and its photoproduct, and hexane-ethyl acetate mixture (20:1) for trans-nonachlor and its photoproduct. Gas-liquid chromatography (GLC) studies were made using a 6-ft x 1/8-in ID glass column packed with 10% DC-200 (viscosity grade 12,500) on 80/100-mesh Gas-Chrom Q with an injection temperature of 225°C and a column and detector temperature of 200°C. Retention times, in minutes, with a nitrogen flow rate of 60 ml/min for the designated compounds were: I - 9.1; II - >120; III - 12.3; IV - 12.0; V - 19.4; VI - 10.7; VII - 24.2; VIII - 12.7; IX - 20.8. Hexadeuterodimethylsulfoxide was the primary solvent used for the proton magnetic resonance (PMR) and ¹³C-nuclear magnetic resonance (NMR) studies; however, pentadeuteropyridine and deuteriochloroform were also used. The toxicity of the compounds was determined on adult female houseflies of the SCR insecticide-susceptible strain and on male white mice of the Swiss-Webster strain. The flies were used 2-5 days after emergence and they were treated topically, on the thorax, with 1.0 µl of an acetone solution of the test compound; mortality determinations were made 48-hours after treatment. Also, toxicity assays were made with flies pretreated with 5 µg of sesamex synergist dissolved in 1 µl of acetone and applied to the thorax 1 hour before application of the test compound. The mice, weighing approximately 20 g, were treated intraperitoneally with 50 µl of dimethylsulfoxide solution of the test compound; mortality determinations were made 48-hours later.

Preparation and isolation of photoproducts. The photochemical apparatus consisted of a 400-watt, high-pressure, mercury-vapor lamp placed in a water-cooled, double-walled, clear, fused-quartz immersion well. The radiant energy reaching the sample was restricted by a pyrex absorption sleeve which blocked radiation below 280 nm. The cyclodienes (about 4 g in 165 ml of acetone) were irradiated for the respective times necessary to achieve reasonable yields of the photoproducts, which were isolated by GLC on Florisil on about 1 column. With heptachlor epoxide, the irradiation was for 2 hours, followed by chromatographic removal of photoproduct II [eluted with chloroform-hexane mixture (2:1)] and recovery of a mixture of unreacted material and photoproduct III [eluted first with hexane-chloroform mixture (4:1)]. The mixture of heptachlor epoxide and photoproduct III was irradiated again for 2 hours, followed by removal of photoproduct II; this overall procedure was repeated two more times in order to accumulate photoproduct II (an intermediate in formation of photoproduct III). Finally, heptachlor epoxide [I] was separated from photoproduct III by taking advantage of the low solubility of photoproduct III in cold ether. [The final yields were: 49% unreacted heptachlor epoxide; 49% photoproduct II (m.p. 178.5 - 181.5°C, from chloroform-hexane); 26% photoproduct III (m.p. 208 - 209°C, from chloroform-hexane).] cis-Chlordanes solutions were irradiated for 48 hours and the photoproduct was separated on Florisil, eluted with petroleum ether for elution and repeating the column purification to obtain pure material (76%, m.p. 148.5 - 151°C, from hexane) and unreacted cis-chlordanes (18%). trans-Chlordanes was converted to its photoderivative by the same

general procedure and the pure product was obtained after one passage through Florisil with petroleum ether (12%, m.p. 168.5 - 170°C, from hexane). (Recovery of unreacted trans-chlordane was 74%.) trans-Nonachlor was photodecomposed in the same general procedure but three passages through the Florisil column were required to separate unreacted trans-nonachlor (70%) from photoproduct IX (6%, m.p. 154 - 155.5°C, from hexane).

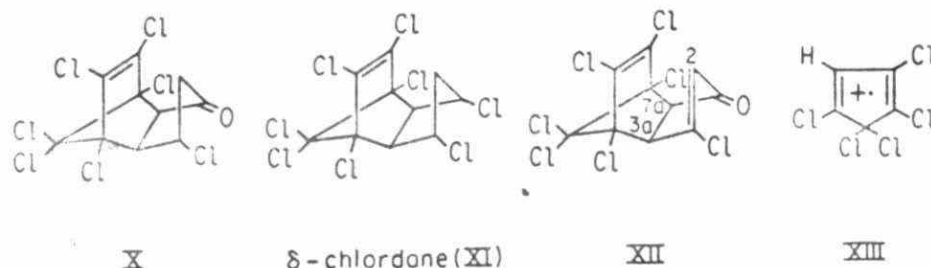
Groups of young bean plants were sprayed to run off with a methanolic solution of the cyclodiene (1% w/v) with or without added rotenone (1% w/v), a potential photosensitizer (5,6). After exposure to sunlight for 4 hours, the plants were rinsed with ether and the rinses were subjected to TLC and GLC analyses.

Results

Photoalteration on plant surfaces. The conversion efficiencies of the designated cyclodienes, exposed on rotenone-treated bean leaves to sunlight for 4 hours, were estimated from GLC and TLC analyses to be as follows: 50 - 60% II and 1% III from I; 15 - 20% V from IV; 15 - 20% VII from VI; 15 - 20% IX from VIII. The photoproducts from the plant surfaces are identical (TLC, GLC, IR, and MS) with those formed in acetone solutions exposed to ultraviolet light. No detectable photoproducts were formed in the absence of rotenone as a photosensitizer; so, the reactions on plant foliage are dependent on the presence of the photosensitizer.

Structure of two photoproducts derived from heptachlor epoxide. One of the heptachlor epoxide photoproducts [II] is a precursor for the other one [III] because photoproduct II converts to photoproduct III (identified by TLC, GLC, IR, and MS) in 70% yield when it is exposed in acetone solution to ultraviolet light for 2 hours. On bean leaves exposed to sunlight, rotenone sensitizes the conversion of heptachlor epoxide to photoproduct II but not the conversion of II to photoproduct III; thus, there is a rapid build-up of photoproduct II from heptachlor epoxide in the presence of rotenone, whereas photoproduct III is formed in significant amounts only after prolonged exposure. Direct exposure of photoproduct II to sunlight on plant foliage results in its slow conversion to photoproduct III, but a small amount of photoproduct II remains, even after 7 days.

Photoproduct II contains a ketone group (IR) and it is isomeric with heptachlor epoxide and photoproduct III (elemental analysis, MS). One possible isomeric structure [X] is not appropriate because neither of two epimeric chloro compounds formed on reduction of photoproduct II to an alcohol, followed by chlorination, is the same as δ -chlordane [XI]. However, the spectral properties (IR, PMR, 13 carbon-NMR, MS), of each of these derivatives and of the photoproduct itself, are consistent with the designated structure for photoproduct II. In particular, three protons (H_2 , H_{3a} , and H_{7a}) of photoproduct II have similar chemical shifts and splittings in the PMR spectrum to those of



the corresponding protons of the related ketone [XII] of known structure (7); also, the IR and UV spectral absorptions due to the α,β -unsaturated ketone grouping are similar for the two compounds. The hexachlorocyclopentane moiety in photoproduct II is clearly indicated by an AB pattern in the PMR spectrum due to two protons (H_5 and H_6) and by a prominent pentachloro-ion [XIII] (m/e 236) in the MS of the alcohol and two chloro derivatives. The ^{13}C NMR spectrum of photoproduct II is also consistent with the structural assignment; it shows resonances at the following δ values downfield from tetramethylsilane: 57.7 and 62.3 ppm, each resulting from two ^{13}C carbon nuclei; 75.7, 76.2, 99.9, 138.2, 165.3, and 198.6 ppm, each due to single ^{13}C carbon nuclei.

Photoproduct III is identical to the compound previously described (1,2), for which structure IIIA (1,2) or structure IIIB (1) has been proposed. Structure IIIB is preferred for photoproduct III because proton H_6 resonates at relatively higher field in the PMR spectrum of the photoproduct than in spectra of related half-cage structures whereas the corresponding signal for the alcohol derived by NaBH_4 reduction is at lower field. This is in accord with the anisotropies of the carbonyl and hydroxyl groups. The structure designated [IIIB] for the photoproduct is also preferred to the alternative structure [IIIA] on the basis of a possible intramolecular hydrogen transfer mechanism in the conversion of photoproduct II to photoproduct III.

Nature of photo-trans-chlordane [VII] and photo-trans-nonachlor [IX]. Photo-trans-chlordane is an isomer of trans-chlordane and photo-trans-nonachlor is an isomer of trans-nonachlor (elemental analysis, MS). In each case, a typically low field singlet in the PMR spectrum and the lack of IR absorption due to a disubstituted dichloroethylene grouping indicate that the photoisomers have half-cage structures. Formation of these two photoisomers proceeds slowly relative to the rate of photobridging encountered with cis-chlordane, possibly because a less-accessible hydrogen needs to be abstracted for bridging with the trans compounds; bridging to the central carbon of the cyclopentane ring is precluded by the endo-configuration of the chlorine substituent (1). The PMR spectrum of photo-trans-nonachlor shows it to have structure [IX]. Photo-trans-chlordane gives a closely related PMR spectrum which establishes the similarity of these two photoproducts and, in addition, shows the presence of a methylene group; so, in photo-trans-chlordane, the bridging occurs as shown

in structure [VII].

Toxicity of the photoisomers. The two photoisomers [II and IIIB] of heptachlor epoxide differ markedly in their toxicity to houseflies and, to a lesser extent, mice (Table 1). It is

TABLE 1
Toxicity of Cyclodienes and Photoisomers

Compound (See Fig. 1)	LD ₅₀ , Mg/Kg		Mouse
	Housefly		
	Without Synergist	With Sesamex Pretreatment	
Heptachlor epoxide [I]	7	7	18
Photo-heptachlor epoxide [II]	>2500	>2500	36
Photo-heptachlor epoxide [IIIB]	2	1	6
<u>cis</u> -Chlordane [IV]	15	15	30
Photo- <u>cis</u> -chlordane [V]	32	28	20
<u>trans</u> -Chlordane [VII]	225	250	130
Photo- <u>trans</u> -chlordane [VIII]	>2500	>2500	>1000
<u>trans</u> -Nonachlor [VIII]	75	90	>500
Photo- <u>trans</u> -nonachlor [IX]	550	425	-

interesting that the two-step pathway for heptachlor epoxide photoisomerization shown in Fig. 1 initially involves conversion of the toxicant to an intermediate [II], which has a reduced toxicity to mice and which is completely nontoxic to houseflies, and then to the end product [IIIB], which is more toxic to flies and mice than the parent heptachlor epoxide [I]. Photo-cis-chlordane [V] is approximately half as toxic to flies as the parent cyclodiene but it is slightly more toxic than cis-chlordane to mice. Photo-trans-chlordane [VII] and photo-trans-nonachlor [IX] are essentially nontoxic. Pretreatment with sesamex has little or no effect on the toxicity of these compounds to houseflies (Table 1).

Discussion

The results reported here establish several novel types of

phototransformations for methano-bridged cycloalkene insecticide chemicals. Heptachlor epoxide converts to a compound with an α,β -unsaturated ketone grouping and a dichloroethane moiety, possibly by intramolecular transfer of two hydrogen atoms. The two new functional groups generated allow bridge formation through transfer of one hydrogen atom to yield the half-cage structure. To the best of our knowledge, comparable photochemical reactions have not been reported previously for related compounds. A portion of the final photoisomer yield may arise from photoreaction(s) of heptachlor epoxide not involving the identified intermediate, but the studies were not designed to test this possibility. The transformations occurring with trans-chlordane and trans-nonachlor appear to be novel, also, because the half-cage products contain a four-membered ring system rather than the five-membered ring system present in the half-cage products derived from related compounds.

The photoisomers of heptachlor epoxide, cis-chlordane, trans-chlordane, and trans-nonachlor are probably of little importance in evaluating the significance of environmental residues associated with the use of heptachlor and chlordane as insecticide chemicals. The photoisomers are not expected to form in appreciable amounts in the environment unless a potent photosensitizer is present. Further, these studies suggest that the small quantities of photoisomers which might occur do not pose a hazard, from a toxicological standpoint.

Acknowledgment

This research was supported, in part, by grants from the U.S. Public Health Service (grant ES 00049), the U.S. Atomic Energy Commission [Contract AT(40-1)-34, Project 113], and The Rockefeller Foundation. The authors thank Louis Lykken, Judith Engel, Eugene Bellet, and Sarjeet Singh of this laboratory for valuable assistance and advice during the course of these studies. They give special thanks to Charles Reilly and Dick Willson, Shell Development Co., Emeryville, Calif., for assistance in the magnetic resonance studies.

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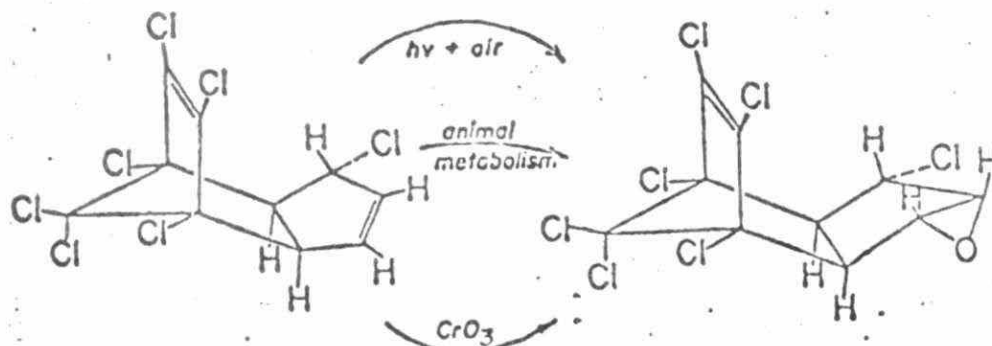
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PHOTO-DECOMPOSITION PRODUCTS OF HEPTACHLOREPOXIDE

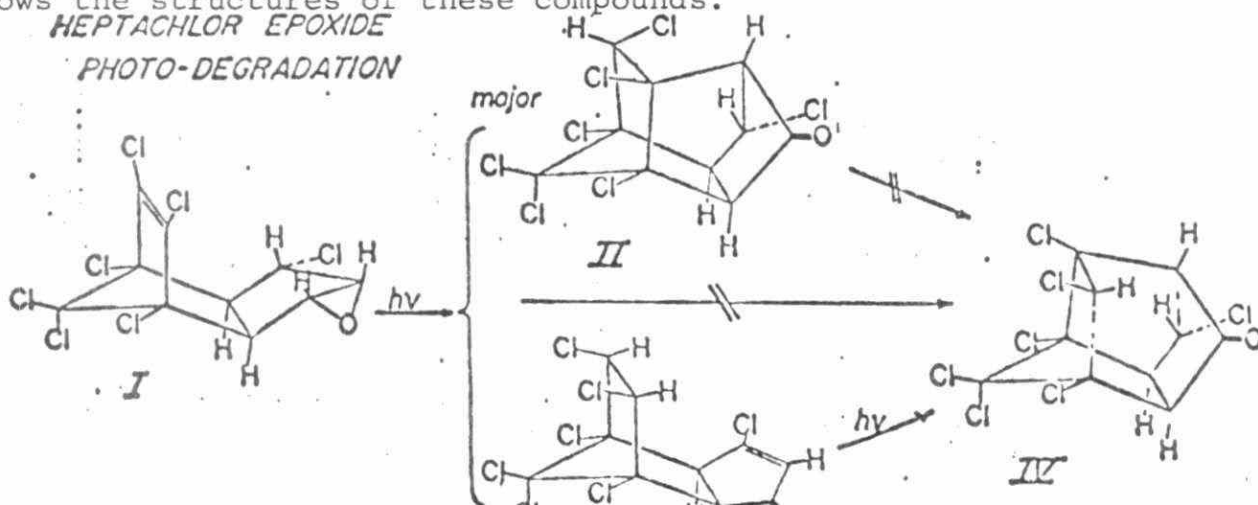
Charles F. Hammer
Department of Chemistry
Georgetown University

Heptachlor epoxide is a metabolic and environmental oxidation product of a widely used pesticide, heptachlor, as shown below.

HEPTACHLOR CONVERSION TO HEPTACHLOR EPOXIDE



The epoxide is about three times more toxic than the original pesticide (see Table 1). It is unstable to sunlight, being converted in about 90% yield to a stable isomeric ketone, II, that is about seven times still more toxic, or approximately twenty times more toxic than the original heptachlor. The remaining 10% is converted to an unstable (and relatively nontoxic) unsaturated ketone, III, which on further irradiation is converted to a third isomeric ketone, whose structure may be IV. No toxicology studies have been done on IV yet. The following figure shows the structures of these compounds.



A summary of the toxicity of heptachlor and its degradation products is shown in Table 1.

Table 1

Toxicities of Heptachlor and its Derivatives*

	LD ₅₀ , mg/Kg	
	housefly (applied with Sesamex)	mouse
Heptachlor (II)	22*	--
Heptachlor Epoxide (I, HE)	7	18
Heptachlor Epoxide Ketone (II, HEK)	1	6
HE Intermediate Ketone (III HEI)	> 2500	36
HE Isomeric Ketone (IV, HEIK)	?	?

*G. W. Ivie, et al., J. Bull Environ. Contam. Toxicol., 7, 376 (1972)

**Converted from % kill data on II and HE given by E.P. Lichtenstein et al., J. Agr. Food Chem., 18, 100 (1970)

Previous workers have observed that I was unstable to UV-light irradiation. Fishler and Korte (Tetrahedron Letters, 32, 2793 (1969) isolated one ketone and suggested II (where C₈CI and H are inverted) as a possible structure based on the known photo products of other similar type pesticides. In another report Benson et al. (J. Agr. Food Chem., 19, 857 (1971), suggested the product to be either II (C₈CI and H inverted) or IV, based on the spectral data.

In an independent study, R.E. Graham, R. Burson, C.F. Hammer, L.B. Hansen and C.T. Kenner (publication submitted) isolated II in about 90% yield and III in about 10% from GLC of the UV-light irradiation of I in solid KBr. They further showed that III is unstable to further irradiation and goes to IV.

This paper will present the NMR data that provide the final proof for the absolute structures of II and III. As yet we have not obtained sufficient amount of IV for good NMR spectra.

A brief summary of other spectral data is in order. The mass spectra of compounds I through IV show parent ions at m/e 386 showing the isomeric formula of $C_{10}H_{15}OCl_7$. The UV spectra of II and IV are consistent with the $n \rightarrow \pi^*$ transition of a saturated ketone while the UV spectrum of III is correct for an α, β -unsaturated, 5-membered ring ketone having a β -substituent.

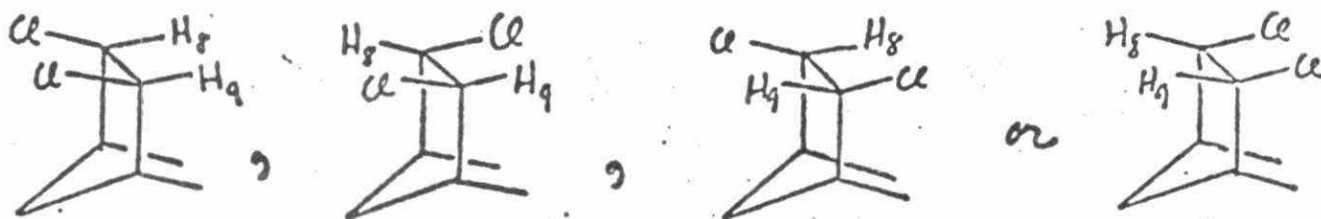
The IR spectra, of II, III and IV in KBr show three different types of carbonyl stretching frequencies, as shown in Table 2.

	$\nu_{C=O}$	$\nu_{C=C}$	<u>Interpretation</u>
II	1775 cm^{-1}	--	5,5-bicyclic ketone
III	1700	1675	α, β -unsat'd 5-ketone
IV	1745	--	5,6-bicyclic ketone

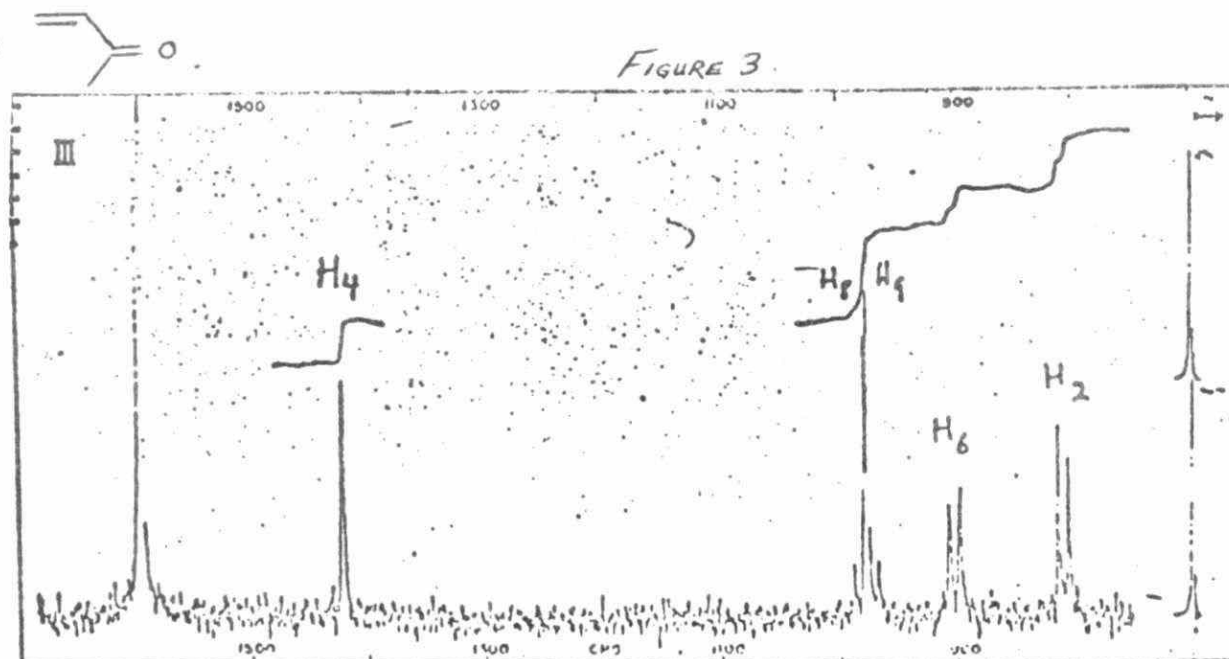
If we assume that the oxygen of the epoxide function of I will probably remain at either positions 3 or 4, then we can surmise that II and IV are probably bridged structures as previously suggested. The problem, however, becomes one of proving which product has the structure corresponding to II or IV and what are the absolute stereo-chemistries of the hydrogens of each.

Let us deal with the simplest structure first. The most reasonable structure for III is the one presented in Figure 2. Note that the overall conversion of I to III represents an internal oxidation-reduction where the hydrogens located on C_3 and C_5 of I are transferred to the original double bond of $C_8 = C_9$. The NMR spectrum of III confirms this structure but does not prove the stereochemistry of II_8 and II_9 . Further high resolution work is required.

Figure 3 shows a single scan of 4.0 mg (our total sample) run on an HR-220 MHz NMR spectrometer. The peak at low field is the impurity solvent ($CHCl_3$ in $CDCl_3$) peak. The doublet ($J = 2H$ z) at 6.4 ppm is assigned to II_4 . II_8 and II_9 are an AB quartet ($J = 9+H$ z) at 4.47 and 4.43 ppm. H_6 is a doublet ($J = 2H$ z) of a doublet ($J = 9-H$ z) at 4.11 ppm and II_2 is a doublet ($J = 9-H$ z) at 3.68 ppm. While the overall structure is confirmed it is not possible to determine which of the four possibilities for H_8 , H_9 is correct.

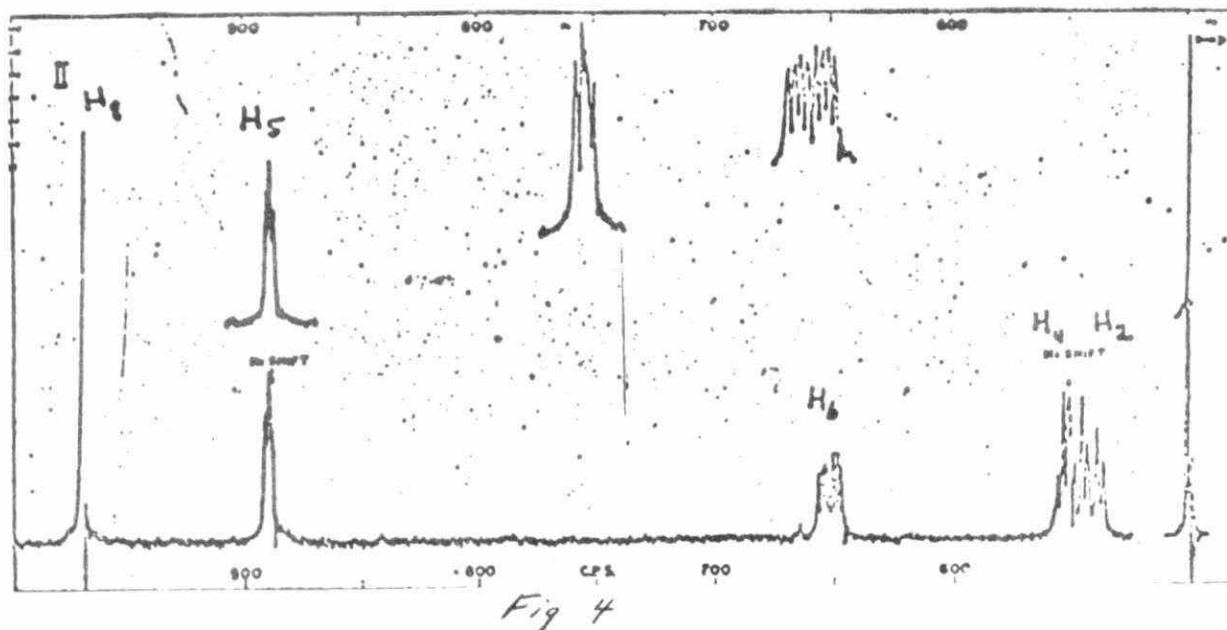


Although one can argue the $J = 9$ Hz means cis, and that since there is a chemical shift difference of H_8 and H_9 , they must be located on the side closest to the anisotropic effects of the



The NMR spectral studies of II are much more satisfying because both the 4,9 location of the bridge and the absolute stereochemistries of the hydrogens can be proven.

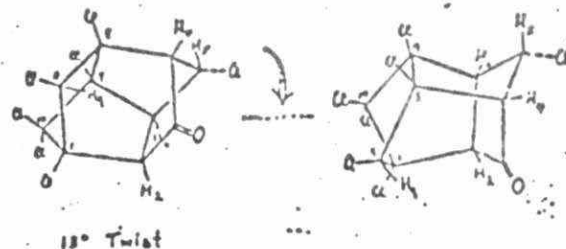
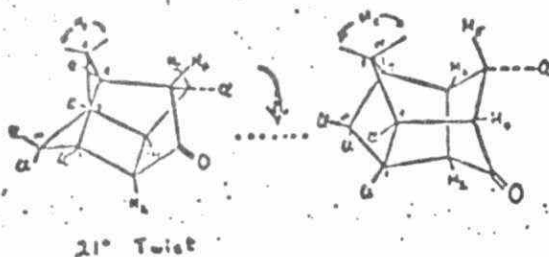
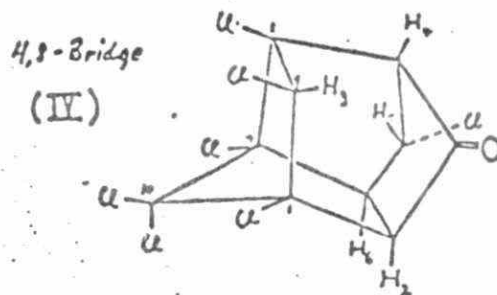
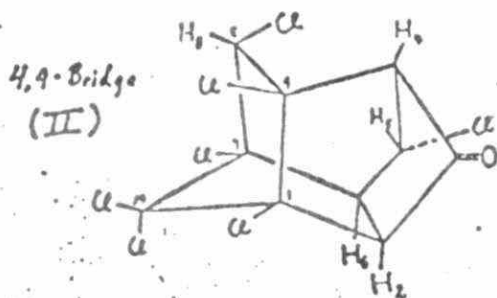
A spectrum of II in CDCl_3 gave four peaks in which the H_8 and H_5 hydrogens were overlapping, even at 220 MHz. The spectrum in C_6D_6 , however (shown in Figure 4), was shifted with H_8 and H_5 now 0.36 ppm apart. H_8 turned out to be a singlet at this resolution and H_5 was now a doublet of a doublet with J's of 2.2 and 1.7 Hz.




H_6 was an 8-line pattern of a doublet of a doublet of a doublet with J 's of 6.0, 2.6, and 1.7 Hz. Partial decoupling of H_5 removed the 1.7 Hz coupling. H_2 was assigned to the highest field hydrogen because it was a doublet of a doublet with J 's of 6.0 and 2.3 Hz. This left H_4 as a simple quartet of $J = 2.3$, which can be explained only if H_4 is coupling to three other hydrogens with similar coupling constants. Indeed, the extra couplings were found in H_5 (2.2), H_6 (2.6) and H_2 (2.3). As the resolution at 220 MHz was only about 1.2 Hz, these couplings would not be resolved. Two of these are virtual-type coupling to which we will return later.

But which bridged structure is correct and what is the configuration of the H_8 in II or H_9 in IV? An examination of Dreiding models (drawn below) reveals that if II is correct and H_8 is inside the "cage" then H_5 and H_8 are located about 1.1 Å apart and H_8

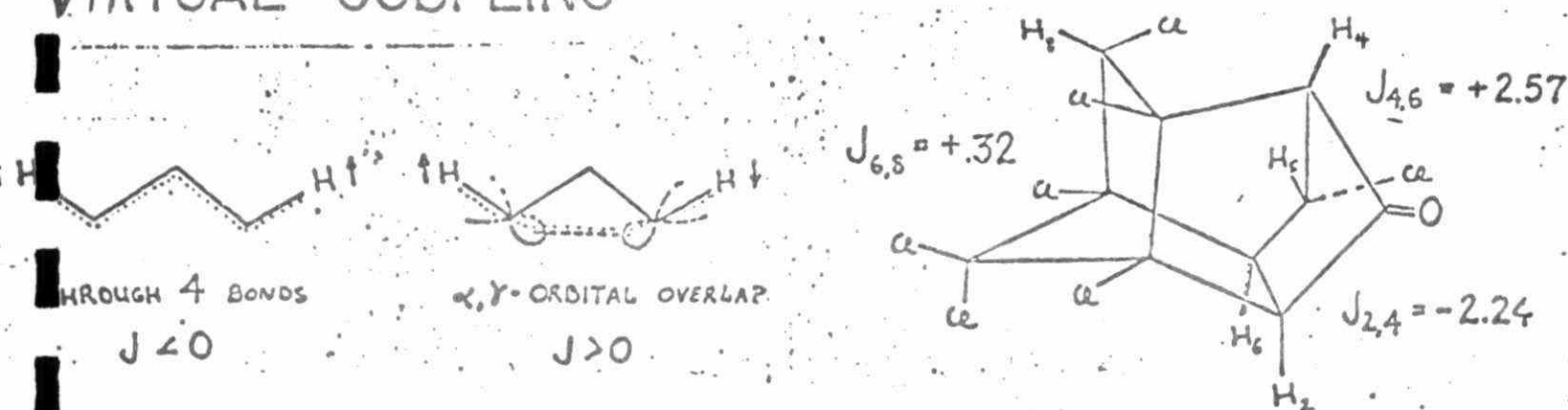
should give a 40 to 45% NOE enhancement when H_5 is irradiated.



D Decoupling on the 220 instrument is difficult, hence a 100MHz instrument was used. While H_4 and H_2 now overlapped H_8 and H_5 were far enough apart (36 Hz) to make the experiment possible. No effect was observed, however, and the experiment was negative. This result could mean that structure IV is correct, although negative NOE's do not offer conclusive evidence. Moreover, the much higher resolution of the JEOL 100 MHz instrument showed that H_8 was not a singlet but was instead a doublet with $J = 0.32$ Hz, another "virtual coupling".

The commonly accepted explanation of virtual coupling requires a  or "zigzag" configuration of the two hydrogens, in which the bonds are essentially coplanar, as shown below.

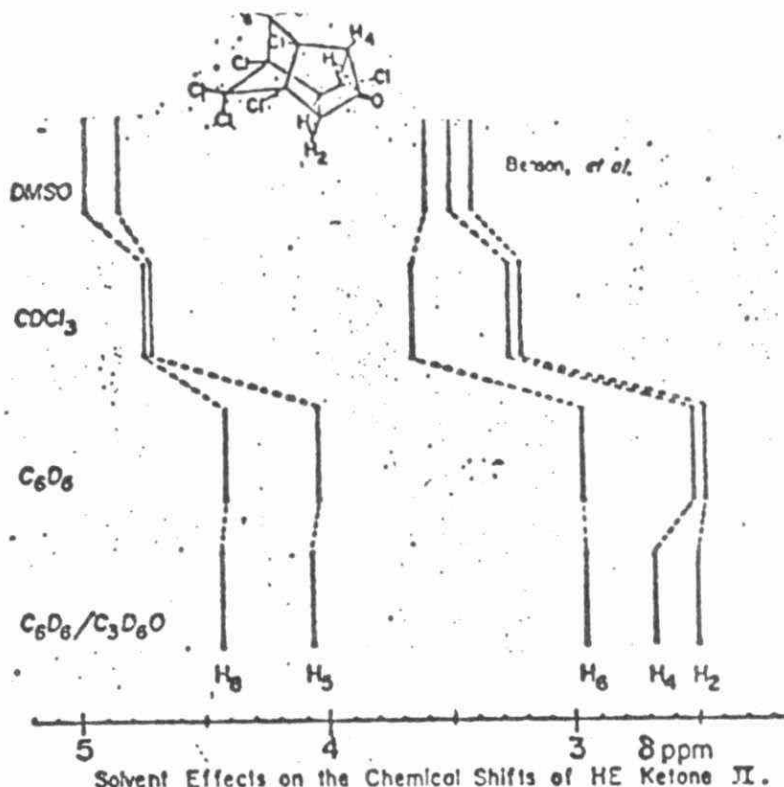
VIRTUAL COUPLING



Thus H_8 could be virtual-coupled only if it is located outside the cage (one would not expect an NOE effect here). The problem reduces to a simple one. If II is correct H_8 must be coupled to H_6 , whereas in IV it would be coupled to H_2 . Yet at 100 MHz, H_2 is not resolved from H_4 in C_6D_6 or in $CDCl_3$. In addition, we had only a 5 mg sample left. Resolution was made possible by a fortunate circumstance. I was in Tokyo at that time and was permitted to use JEOL's new bread-boarded 1H -ET system with a time-shared decoupling capability.

I prepared the sample in a microtube, left it with the technician, and left to run spectra on another instrument. Finding that the microtube would not work, he replaced the sample in another tube, mistakenly diluting it with d_6 -acetone, I was grateful for this mistake, since even at 100 MHz H_4 and H_2 were completely resolved. A summary of these interesting solvent shifts is shown below. (next page)

The 1H - FT spectrum also contained a small water peak, which was useful as an internal standard. The computer size limited the resolution to 0.8 Hz, not good enough to resolve the H_8 0.32 Hz doublet. Even so, decoupling of H_6 should increase the peak height of H .



Unfortunately all the other peaks were also coupled to H_6 , so that only the water peak could be used for comparison. Prior to decoupling the $H_8:H_2O$ ratio was 3:1. Decoupling of H_6 increased the ratio to 4:1, thus showing that II is the correct structure for the major photo-degradation product of heptachlor epoxide. In added proof, all the expected changes of the other hydrogens occurred, making a final confirmation of the absolute stereochemistry of II.

Finally, the data (δ 's, J 's, and line frequencies) were iterated in a 5-spin LAOCOON-III calculation to give the data in Table 3. Even when some of the signs of the J 's were reversed, the program iterated to the signs shown. Thus two of the virtual couplings, $J_{6,8}$ and $J_{4,6}$, are positive and $J_{2,4}$ is negative.

As yet we do not have a good NMR spectrum of IV. If it has all the couplings observed in II it will be interesting indeed.

(Table 3 on next page)

Table 3

HEPTACHLOREPOXIDE KETONE (II)

 $^1\text{H-FT}$ line width 0.8 Hz

Chemical Shifts*		Coupling Constants (± 0.07 Hz)**	
δ_2	3.097	$J_{2,4}$	-2.24 Hz
δ_4	3.272	$J_{2,5}$	-0.02
δ_5	4.668	$J_{2,6}$	5.96
δ_6	3.557	$J_{4,5}$	2.20 (2.41)***
δ_8	5.020	$J_{4,6}$	2.57
		$J_{5,6}$	1.68 (1.46)***
		$J_{6,8}$	0.32
$J_{2,8} = J_{4,8} = J_{5,8} = 0.00$ Hz			

*Relative to $\delta_{\text{C}_6\text{HD}_5}$ of the ca. 60:40:: C_6D_6 : $\text{C}_3\text{D}_6\text{O}$ solution with 4.6 mg in ca. 0.5 ml.

**Iterated values obtained when the broad center peak of H_5 was weighted 0.1.

***Values obtained when $J_{6,8}$ started as -0.32 Hz (note that it iterated to +0.32).

JAN 17 1973

S. H. Allen

Find pesticide breakdown by-products 22 times as toxic

By William Hines
Sun-Times Bureau

WASHINGTON — An insect killer used by the millions of pounds each year in American agriculture has been found to break down into by-products that are up to 22 times as poisonous as the original chemical.

The substance, called heptachlor, is transformed by the action of sunlight into more potent materials, some of which have been detected as residues on root crops such as potatoes and carrots. Feeding experiments on rats have shown that death from liver damage can follow overdose of these

secondary chemicals.

Heptachlor has been in use for about 25 years and is produced in an annual volume of about 6 million pounds in this country. It represented about 2 per cent of the pesticide output of the agricultural chemicals industry in the year that ended last June 30.

Chemist Charles Hammer of Washington's Georgetown University told of new discoveries about heptachlor's by-products at a regional meeting of the American Chemical Society.

After the material is sprayed on cropland it is converted, largely by solar energy, into a chemical called heptachlor

epoxide, which is a little more than three times as toxic as the original material. Further action of sunlight creates a mixture of subsidiary compounds, 90 per cent of which are heptachlor epoxide ketone (HEK).

Hammer said HEK is so toxic that 1 part per million in the

environment will kill a house fly and 6 parts per million a mouse. (These are so-called "LD-50" figures, which signify the dose which will prove lethal to one-half of a given population of creatures.)

Neither Hammer nor two other scientists who appeared with him at a press conference claimed any knowledge of harm done to human beings by heptachlor or its by-products but added that "a toxicologist might take issue with this statement."

Hammer said that in his judgment the arguments now entered against the widespread use of DDT because of known or assumed health hazards might logically be applied to heptachlor as well. He did not say flatly that he would advise banning it, however.

Carroll Collier of the Environmental Protection Administration said that a study group empaneled by EPA Administrator William D. Ruckelshaus has completed work on a heptachlor survey and has sent its recommendations to Ruckelshaus' aides.

Collier said he did not know what recommendations were made or when and how they would be acted upon.

Pesticide termed more toxic than DDT

WASHINGTON (AP) — A chemist reported yesterday that heptachlor, a still relatively widely used agricultural pesticide, changes in sunlight to a stable substance that is 22 times more toxic than the original compound.

Dr. Charles Hammer of Georgetown University reported finding the first proof of this housefly, mice and other animal tests.

He said the newly uncovered substance is "considera-

bly more toxic than DDT," which has been banned from the market in the United States as potentially hazardous to man and animals.

Meanwhile, an Environmental Protection Agency spokes-

man said some restrictions on the registered use of heptachlor and another still relatively widely used pesticide, chlordane, will soon be recommended to the Government by a special committee of EPA scientists.

RESIDUES OF CHLORDANE AND RELATED PRODUCTS

by

J. R. Elliot

Chief

Food Inspection Division

Ontario Region

November 15th, 1972

The Health Protection Branch of the Department of National and Welfare have done some studies on the total Canadian diet. These studies are available and if the committee wishes, can be made available to it. The program is under the direction of Dr. Mannell who is the toxicologist with the Food Advisory Bureau, Food Directorate, NH & W.

The second comment I would make is that our role is that of a regulatory agency and as such, we tend to go where we think problems may exist. Thus there is a bias in our sampling techniques. I should also like to point out that we have a responsibility for imported products as well as domestic ones. In the past three or four years, we have put a great deal of effort into looking at imported products of one sort or another and our results tend to be rather spotty, because of the large number of products involved.

What we have done is to pick from our data a period in time shortly after heptachlor was banned and then a second period quite recently some significant time after the use

of heptachlor had been discontinued. We have a computerized print-out system and it is somewhat of a scramble to pick out this sort of information - but it can be done.

The first period selected was October 1st, 1969 to March 31st, 1970. This is the reporting time of the laboratory. Thus the samples would have gone into the laboratory considerably before the October 1st date. On dairy products in that six-month span, we had 44 samples analyzed. Twenty-seven of these contained some heptachlor epoxide. Seventeen were negative. The range in residues of heptachlor epoxide was from 0.01 ppm to 0.1 ppm. The overall average on the total 44 samples was 0.008 ppm. I should point out that these figures, although they are collected from the computerized print-out that contains analyses from all sorts of areas, the figures presented here represent Ontario products. During this same time period, we had 68 analyses of vegetables and all were negative for heptachlor epoxide.

In fruit products 22 samples were analyzed; 6 showed residues of heptachlor epoxide which ranged from 0.001 to 0.01 ppm. Sixteen samples were negative. During this six-month period we found no residues of chlordane on any of the products examined.

The second time period selected was July 1, 1971 to December 31, 1971. This would be after the ban on the use of heptachlor. On dairy products a total of 56 samples, 12 were positive with the range 0.01 to 0.06 for heptachlor epoxide and 44 were negative. The overall average was 0.003. It should be noted that this is down from the 0.008 average in the earlier sampling period. During this same period, 31 analyses were read on meat products, 2 showed a low level of heptachlor epoxide (0.01 and 0.02 ppm). Twenty-nine samples were negative. These residues, it should be pointed out, are on a fat basis, not on a whole product basis. In this same period, 196 vegetable samples and 86 fruit samples were collected and analyzed and all were negative for both heptachlor epoxide and chlordane.

These figures have not been analyzed in any way to assess their significance. The overall conclusion that one could reach is that the residues in the latter period of time are down somewhat from those of the earlier sampling period. Perhaps a general comment is in order with respect to the identity of the products sampled. I cannot indicate, specifically, for each sample, although this would be available in our files. However, I believe it is fair to state that most of the vegetables would be from southwestern Ontario and the Holland Marsh area. The dairy products would be from all over the province.

PESTICIDE RESIDUES IN FEED

by

R. F. Knapp

November 15th, 1972

There has been limited sampling of feeds for pesticide residues over the past several years. A few residues have been found on feed materials rather than on the finished feeds.

In 1969, 19 samples were analysed for heptachlor, heptachlor epoxide, and chlordane. The records available are not very specific. On 16 of the samples reported, a notation was added "residues not detected" and on 3, the notation was "detected". I do not know the sensitivity level at which these residues were detected. The three samples were a sample of mixed hay, a sample of dried beef pulp and a sample of meat scrap.

There are no records of samples being tested for these three chemicals in 1970. In 1971, six samples were tested and none were found to contain residues at a sensitivity level of 0.005 ppm. In 1972, 20 samples were checked for residues. Heptachlor was found in one sample of tallow at 0.025 ppm. It is suspected that this residue resulted from contamination, but no adequate explanation of its source was established. The other 19 samples were free of detectable residues.

It should be pointed out that insofar as feeds are concerned, it is usual to check the possible feed components rather than to analyse the finished feeds, for residues. Thus most of the analyses are run on hay, meat meals, fish meals, silages, vegetable scraps, etc. In this way feed ingredients that have harmful pesticide residues can be diverted from the feeding systems.

Provincial Pesticide Residue Testing Laboratory

R. Frank, PhD.

WATER

In almost 6 years of monitoring waters on a random basis, only 1 contained a trace of heptachlor epoxide and only 4 contained chlordane. All four cases of chlordane contamination were found in 1972 and were associated with spraying of soil along the river bank. The residues in water were trace, 0.03, 0.04 & 0.05 ppb (Table 1)

TABLE 1.

Year	Water Analysed	Water containing insecticides		Remarks
		Chlordane (#)	Heptachlor or Heptachlor epoxide (#)	
1967	7	0	0	
1968	3	0	0	
1969	95	0	1	Level of HE was a trace
1970	128	0	0	Sample from water from Chatham fire after passing through sewage treatment plant contained 0.55 ppm chlordane.
1971	209	0	0	
1972	79	4	0	0.04,0.05,Tr,0.03. In 3 cases found that chlordane was present in soil on bank of river.
TOTAL	442	4	1	

MILK SURVEYS

(a) 1967-69 - Some milk produced in the Southern & Western regions of Southern Ontario contained low residues of heptachlor epoxide. The average residue in the southern region was below 0.01 ppm and originated from 2 of the 12 counties. The counties of Lambton and Middlesex had average residues of 0.005 & 0.002 ppm respectively in the butterfat. These counties and the 3 counties bordering them in the Western region had employed

heptachlor for turnip production and for the treatment of cereal grain. Both cull turnips and surplus treated grain had been used as feed for dairy cattle giving rise to heptachlor epoxide in milk. The Western region counties were Bruce (0.008 ppm), Huron (0.023 ppm) and Perth (0.013 ppm).

(b) 1970-71 - Only the Southern region was resampled in 1970-71 and no samples were collected that contained detectable residues of heptachlor epoxide, not even in the counties of Middlesex and Lambton.

TABLE 2 Residues of Heptachlor epoxide in milk from 1967-69 survey

Region of Ontario	Samples (#)	Residue Range (ppm)	Samples containing Heptachlor epoxide (%)	Mean Residue (ppm)
Central	387	N.D.	100	N.D.
Eastern	489	N.D.	100	N.D.
Northern	70	N.D.	100	N.D.
Southern	372	N.D. .01 - .09	96.5 3.5	} <0.001
Western	333	N.D. .01 - .09 .10+	89.2 10.5 0.2	
Ontario	1,651	N.D.	97.0	} 0.003
		.01 - .09	2.9	
		.10+	0.1	

Fats from Domestic Animals

Animals fats have been analysed over the past 4 years and include bovine, porcine, and avian fats & eggs. (Table 3). In less than 5% of 172 animal fats analysed was heptachlor detected. These 5% ranged from a trace to 0.044 ppm in the extractible fat. Only 1 of 68 samples of porcine contained a trace of heptachlor epoxide. Only 4 of 50 samples of avian fat contained heptachlor epoxide, however, one of these samples contained over 1 ppm. The source was never traced. The other three contained from a trace to 0.018 ppm. Only 1 egg of 73 analysed contained residues of heptachlor epoxide, in this case the level was 0.024 ppm. Since the origin of the livestock samples could not be traced it was difficult to determine if they came from a single area. Samples were collected in several locations in the Province. (Table 3)

TABLE 3

Residues of Heptachlor epoxide in Domestic fats.

Fat	Year	Samples Analysed (#)	Presence of Heptachlor Epoxide		Content in extractible fat	
			(#)	(%)	Residue Levels (ppm)	Mean Residue (ppm)
Bovine	1969	108	7	6.5	Trace, .005, .010, .020, .032, .044(2)	0.001
	1970	32	1	3.1	Trace	N.D.
	1971	21	0	0		N.D.
	1972	14	0	0		N.D.
	Total	175	8	4.6		Trace
Porcine	1969	21	1	4.8	Trace	N.D.
	1970	24	0	0		N.D.
	1971	12	0	0		N.D.
	1972	14	0	0		N.D.
	Total	71	1	1.4		N.D.
Avian	1969	13	3	23	Trace (2), .018	.001
	1970	17	0	0		N.D.
	1971	12	1	8.3	1.06	.082
	1972	11	0	0		N.D.
	Total	53	4	7.5		.020
Egg	1969	13	0	0		N.D.
	1970	28	1	3.6	.024	.001
	1971	20	0	0		N.D.
	1972	15	0	0		N.D.
	Total	76	1	1.3		Trace

Human Tissues

The only residues of heptachlor epoxide were found in 1969 when 3 of 10 human milks and 1 of 8 human fats contained detectable residues. For the last 3 years 56 human milk and 210 human fats have been analysed and found to be free of detectable residues.

Fish

From the analysis of several thousand fish, less than 10 contained detectable residues of heptachlor epoxide. Traces were found in white suckers in the Grand River. (Table 4)

TABLE 4 Residues of Heptachlor epoxide in Human Tissues

Tissue	Year	Samples Analysed (#)	Presence of Heptachlor epoxide		Content in extractible fat	
			(#)	(%)	Residue levels (ppm)	Mean residue (ppm)
Human milk	1969	10	3	30	Trace, .08, .09	0.017
	1970	37	0	0		ND
	1971	7	0	0		ND
	1972	20	0	0		ND
	Total	74	3	4.1		0.002
Human fat	1969	8	1	12.5	0.08	0.010
	1970	34	0	0		N.D
	1971	97	0	0		N.D
	1972	79	0	0		N.D
	Total	218	1	>0.5		Trace

Bird Tissues

Following the analysis of 60 species of wildlife birds caught in Ontario only one was found to contain residues of heptachlor epoxide. As illustrated in table 5 heptachlor epoxide was found in the brain tissue of ring necked pheasant shot in the county of Lambton. The mean residue for all eight birds shot in Lambton was 0.017 ppm. While all birds in Lambton contained heptachlor epoxide none was found in birds received from other counties of the province (Table 5)

TABLE 5 Analysis of brains from shot Ring Necked Pheasants 1967-69

Year	Location	Samples Analysed (#)	Samples Containing Heptachlor Epoxide		Residue Levels (ppm)	Mean (ppm)
			(#)	(%)		
1967	Essex	12	0	0		N.D
1968	Essex, Lambton, Lincoln, Welland, York	60	8	13	.009, .012, .014, .015, .019(2), .022, .027*	.0023
1969	Essex, Northumberland	16	0	0		N.D.
	TOTAL	88	8	9.1		.0016

* All samples from County Lambton.

Feed

Following the discovery of residues of heptachlor epoxide in milk in Bruce County (1968) at a level of 0.09 ppm the investigation revealed the feeding of treated grain to dairy cattle. On one farm the farmer was feeding milled grain with 2.60 ppm heptachlor and 0.50 ppm of γ chlordane. The grain going into this feed contained 3.74 ppm heptachlor and 1.01 ppm γ chlordane. This was effectively stopped and residues in milk disappeared. A second feed sample in 1968 from some 3 year old treated grain used in a feed was 0.26 ppm heptachlor and 0.13 ppm HE.

Poisoning

1969 - A 2 1/2 month old calf was treated twice in 1 day with chlordane to control lice. The animal died after convulsions in 24 hours. The symptoms were described as chlordane toxicity.

TABLE 6

Residues of chlordane in a poisoned calf
and extractible fat.

	Extractible Fat (%)	Chlordane (ppm)	Heptachlor (ppm)
Skin	4.11	169	23
Liver	5.04	281	127
Kidney	4.51	612	138
Muscle	3.22	111	45
Brain	13.08	158	78

1971 - Two dogs died of convulsion indicative of insecticide poisoning. However, analysis revealed no harmful levels of any known organochlorine or organophosphorus insecticides. Chlordane was present in the stomach at 0.4 ppm.

Plant Material

Rutabaga. - In 1967-68 samples of turnip were collected from 10 rutabaga producers from Mitchell, Exeter & Bright. Roots from all 10 producers contained heptachlor from a trace to 0.008 and heptachlor epoxide from .005 to 0.020 ppm or a total residue from 0.009 to 0.028 ppm.

TABLE 7 Heptachlor & Heptachlor epoxide residues in rutabagas

Sample (#)		Content of Heptachlor (ppm)	Heptachlor epoxide (ppm)	Total (ppm)
Rutabaga roots	11	.005	.011	.016
Peel	11	.008	.037	.045
Pulp	11	.005	.008	.013

Potatoes

Only one sample of many have been found to contain chlordane or heptachlor. The level of chlordane in the one sample was 0.05 ppm.

Tobacco

Of 51 tobacco samples analysed 4 samples were found with residues from 0.001 to 0.006 ppm heptachlor epoxide in the dried leaf.

Spray Drift

1968 - Spray drift occurred in the city of Toronto when heptachlor was used.

Only trace quantities could be found in adjacent lilac.

1970 - Spray drift occurred when chlordane was used in an urban area. Poplar and maple trees carried from 1.2 to 15.5 ppm chlordane, on fresh weight basis. Strawberries had 1.2 ppm and surrounding grass 1.6 ppm.

1972 - Plant samples collected near a factory revealed residues of chlordane ranged from 0.05 to 3.26 ppm on dried leaves taken from elm, maple, grape, and cattails.

Summary

Heptachlor epoxide has been found in animal products and pheasant in these areas where heptachlor was used in agriculture. The counties of Bruce, Huron, Lambton, Middlesex and Perth appear to have been the major areas of use. Residues of chlordane have not been found in milk, feed or wildlife to date.

USES OF CHLORDANE & HEPTACHLOR

Insects Controlled

Chlordane is used on food and feed crops to control wireworm, white grub, subterranean cutworm, corn root worm, and strawberry root weevil.

Method of Application:

Chlordane is applied to soil as broadcast prior to planting or as a band application at the time of planting or seeding.

Food Crops

Chlordane is used on the following food crops:

- A) Vegetables - cruciferae - radish, broccoli, kale, brussel sprout, cabbage, cauliflower.
- cucurbitae - cucumber and cantelope.
- solonaceae - potato and tomato
- legumes - beans
- others - lettuce, onions, red beets, sweet potato.
- B) Fruit - strawberries.
- C) Cereals - corn.

Food Crop Tolerance

The following tolerances are permitted under the Food and Drug act and Regulations:

<u>Chlordane</u>	0.3 ppm	<u>Fruit</u> - apple, apricots, blackberries, blueberries, cherries, citrus, grapes, loganberry, nectarines, papayas, peaches, pears, pineapple, plums, quince, raspberries, strawberries.
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Vegetables - beans, beets, peas, broccoli, brussel sprouts, cabbage, carrot, cauliflower, celery, collards, cucumber, eggplant, kale, kohlrabi, lettuce, melons, peanuts, peppers, potatoes, radishes, rutabagas, squash, tomato, turnip.

Cereals - corn.

Heptachlor & Heptachlor
epoxide

0.1 ppm Vegetables - beans, cabbage, lettuce, rutabagas

(yellow turnip)

Heptachlor was discontinued for use in Ontario in 1969.

Your file Votre dossier

Our file Notre dossier

 Environment
CanadaEnvironnement
CanadaLands, Forests
and WildlifeTerres, Forêts
et FauneCanadian Wildlife Service
400 Laurier Avenue West
Ottawa, Ontario
K1A 0W1

November 23, 1972

Mr. K.G. Laver,
Ontario Pesticides Advisory Committee,
5th Floor, Mowat Block, Queen's Park,
900 Bay Street,
Toronto 182, Ontario.

Dear Mr. Laver:

At the end of the meeting on heptachlor and chlordane you requested the participants to write their submissions to the Advisory Committee. Since the meeting I have had the opportunity of examining the information which was submitted by Velsicol for their tolerance petition and was unable to find any data on experimental studies on the reproduction of pheasant and quail referred to in the meeting. There was however a reproduction study of both heptachlor and chlordane on hens and a study of transfer of these substances to the egg. With respect to the chlordane study (Volume II p. 142, reference 22) there was no effect on hatchability or growth of the chicks for 28 days. Unfortunately the highest level of feeding was only 0.3 ppm in the diet. It would have been a more convincing study if a level had been selected at which an effect was expected to be found and if an effect level had been demonstrated. The absence of residues of chlordane in the eggs of the egg transfer study (Volume III p. 1375 reference 10) is encouraging but again the feeding level was so low (0.08 ppm in the feed) that it would have been more convincing if several levels had been fed and a level found where there were residues detected. Taking these two studies together, the absence of chlordane residues in the environment of Ontario and the other information in the petition and presentations at the meeting, I am satisfied that the use of A.G. chlordane has probably not created biological effects in Ontario.

The information with respect to heptachlor is not so encouraging. Volume II of the heptachlor tolerance petition submitted by Velsicol contains the reproduction study on chickens and is entitled "Toxicity, residue and reproduction study on heptachlor epoxide-chickens". The study was undertaken by Industrial Bio-Test Laboratories and dated 10 April, 1969. There appears to be a decrease in hatchability among eggs from hens fed the two highest levels (0.1 and 0.2 ppm in the feed). In the table on the viability of hatched chicks one anomaly stand out.

The number of chicks in the control group which died in the first week was 17 out of 75 which hatched, whereas only 6 and 5 out of 76 and 57 died, on the 0.02 and 0.1 ppm feeding level, respectively. This mortality in the controls makes the 19 dead chicks out of 80 hatched in the 0.2 ppm level not significant. I am therefore not satisfied that 0.2 ppm in the food of hens does not have an effect on viability of hatched chicks in the first week. I have been unable to find the residue data which was performed on the eggs and carcasses from this study. Again it would have been more convincing if an effect level had been demonstrated by choosing a dietary feeding level where effects might be expected.

In another study in the submission (Volume III page 918) it appears that the residues in the fat of adult hens at 21 weeks are 10 times higher than the feeding levels and that the levels in the eggs are comparable with the feeding levels. The study however does not show that the levels at 21 weeks are the maximum that would be stored, thus birds in the environment may have a concentration rate higher than 10 times.

I should like to review some of the residue data which have been collected from the Great Lakes for fish eating birds. Dr. Lincoln Reynolds of the Ontario Research Foundation has found heptachlor epoxide in the eggs of Common Terns from Hamilton Harbour. As I mentioned at the meeting these birds are obtaining this substance from the vicinity of the colony since the eggs laid later in the season (to replace lost broken or unhatched eggs) are higher than those amongst first laid eggs. I have calculated a regression equation for the line of best fit to describe the rate of uptake of H.E. with time. It appears that the terns arrive with essentially nothing and after 100 days the eggs contain 0.15 ppm on a wet weight basis. Residues in the adult were undetectable in the breast muscle on arrival but were 0.20 ppm after 70 days. We have found 0.27 ppm H.E. in an egg of a Black-crowned Night Heron from Pigeon Island, eastern Lake Ontario. While this contamination may have resulted from the wintering grounds, this species in Lake Erie did not have detectable residues. In Ring-billed Gulls the highest values were from a colony on Toronto Island and were 0.23 and 0.21 ppm. Other colonies ranged from 0.03 to 0.07 ppm. In Herring Gulls very high values were found in eggs of gulls from eastern Lake Ontario ranging from 0.23 to 0.75 ppm. Western Lake Ontario and eastern Lake Erie had 0.13 to 0.21 ppm and western Lake Erie 0.05 ppm. In Lake Huron values ranged from 0.12 to 0.52 in eggs from the Parry Sound region. The situation with Common Terns is comparable but with values about 10 times smaller ranging generally from 0.01 ppm to 0.11 ppm with occasional high values up to 0.34 ppm.(Toronto Island).

On page 95 of Volume II of the Velsicol submission there is the sentence "The current use of heptachlor as a soil insecticide does not allow it to be available for exposure to wildlife". I would submit that the vaporization of heptachlor from the use of technical chlordane may cause its translocation in air masses, redeposition in rainwater, biological magnification in food webs and deposition into eggs of aquatic birds where it may cause biological effects such as embryonic mortality, failure to hatch or reduction of chick viability. Further I would submit that the levels presently found in the eggs of fish eating birds in the Great Lakes are comparable with the levels at which these effects may be found and are, in some cases up to four times higher than the levels which have been tested by Industrial Bio-Test. There is no information on interspecific variation in susceptibility to this substance. Chickens may be appreciably more tolerant than species in the environment.

I wish to restate my position. AG chlordane does not presently have any information, which I have reviewed, which leads me to recommend its cessation of use. Technical chlordane contains an unacceptable chemical; heptachlor. The concentrations of organochlorine substances which are presently in the Great Lakes environment are so great that in certain locations there are biological effects on indigenous bird species. This situation is unacceptable. The continued use of a comparable substance such as heptachlor which may enter the Great Lakes is inappropriate.

I shall be absent from my office until January 4, 1973 but should be pleased to discuss any details of this letter with you or the committee after that date.

Yours sincerely,

A handwritten signature in cursive script, reading "Michael Gilbertson".

Michael Gilbertson,
Wildlife Biologist,
Toxic Chemicals Section.

Section 4

Residues and Degradation Products of Technical Heptachlor in Various Soil Types¹

FAIRIE LYN CARTER and CHARLES A. STRINGER²

Southern Forest Experiment Station, USDA Forest Service, Gulfport, Miss.

ABSTRACT

The persistence and degradation of heptachlor varied considerably by location in tests in 5 areas of the United States. Relatively high values for 1-hydroxy-chlordane, representing approximately 60% of the insecticide in the soil, were obtained for extracts of a Quincy loamy fine

sand from Oregon. Significant amounts of 1-hydroxy-chlordane were found in the extracts of Lakeland sand from Florida. Generally, heptachlor epoxide represented only a small fraction of the insecticide present in the soils.

Aldrin, chlordane, dieldrin, and heptachlor are currently being compared for their control of various species of termites in different soils and climates in the United States (Beal and Carter 1968). Since effective control of termites requires a persistent insecticide that remains where applied, residues of the pesticides are being checked annually. This paper reports the persistence and degradation of technical heptachlor after field weathering for up to 3 years.

METHODS AND MATERIALS.—Field Test.—Samples for analysis were taken from 5 locations selected to represent major soil types and rainfall patterns in portions of the United States where subterranean termites are a major problem. The soil types at the locations are: Lakeland sand at Marianna, Fla.; Makalapa clay at Honolulu, Hawaii; Lebanon silt loam, Salem, Mo.; Quincy loamy fine sand, Hermiston, Ore.; and Catulpa loamy sand, Union, S. C. The installation procedures, soils, and locations have been previously described (Beal and Carter 1968). The Bouyoucos hydrometer method was used to estimate particle size of soils sampled at the 5 locations (Forest Soils Committee of the Douglas Fir Region 1953). Corrections were not made for organic matter, which was low for all soils except the sample from Hawaii. Organic matter was estimated by the modified Walkley and Black method, a rapid dichromate oxidation method (Forest Soils Committee of the Douglas Fir Region 1953). Soil pH was determined electrometrically. Table 1 summarizes selected properties of a sample of each soil. Considerable differences in appearance were observed for samples taken at different depths and at different plots at certain locations. Thus, we are cognizant of differences in soil properties of samples taken for analysis from the test plots.

The effectiveness of soil treatments against termites is being evaluated by the ground-board test (Johnston 1960), which simulates soil treatment prior to pouring concrete slabs. For the present study, soil samples were taken from 2-ft² plots to which water emulsions of 0.5% technical heptachlor were applied at a rate of either 1 or 2 pints/ft². Samples were usually taken in 6-in. squares from the upper 1-in., 1- to 2-in., and 2- to 4-in. layers. Soil was sampled from 3 of the 10 treatment replicates.

Analysis.—A sample of sieved soil equivalent to

100 g oven-dry weight was extracted for 2 hr on a shaker with 300 ml of a solvent mixture of 2:1 hexane and isopropyl alcohol. A 225-ml aliquot of the filtered extract was then washed 3 times with 75 ml of distilled water. The hexane layer was filtered through anhydrous sodium sulfate, adjusted to 150 ml with glass-distilled hexane to compensate for evaporation loss, and stored in the refrigerator until it was analyzed. Two ml of extract were equivalent to 1 g of the soil.

The aforementioned extraction method has been used routinely since 1965 in this laboratory for field soils involved in termite tests. An estimation of insecticide recovery was ascertained by adding a standard mixture of heptachlor, gamma-chlordane, 1-hydroxy-chlordane, and heptachlor epoxide to 100 g air-dried soils from the 5 field locations. The soils were again air-dried and extracted in accordance with the same procedure. An additional sample of the standard mixture was diluted with the proper amount of hexane and isopropyl alcohol and carried through the same procedure. The estimated percentages of recovery of the 4 insecticides for the 5 soils were never less than 95%. Since extraction efficiencies of solvents have been shown to be affected by soil moisture, air-dried soils should not be fortified for measurement of true recovery rates from field samples extracted in the air-dried state (Saha et al. 1969a). Although many factors in addition to soil moisture can affect insecticide extraction from soils (Saha et al. 1969b), we believe that we can generally compare the results from our various test soils.

Extracts were analyzed with a Micro-Tek³ 2000 MF gas chromatograph equipped with a 130-mc tritium electron capture detector and operated with a pulse power supply. The columns were 6-ft × 1/4-in. glass tubing packed with 3% DC-200 on 100/120 mesh Gas Chrom Q. Operating temperatures and gas-flow rate varied somewhat, but appropriate standards were run daily with the samples. Typical operating parameters were: oven 195°, detector 205°, inlet 215°, and outlet 240°C. The flow rate of the carrier gas, a mixture of 95% argon and 5% methane, was normally 120 ml/min.

Columns packed with 3% SE30 on 60/70 mesh Chromport XXX and 11% (QF-1 plus OV-17) by weight on 80/100 mesh Gas Chrom Q, were used to support the identification of gas chromatograph peaks believed to be 1-hydroxy-chlordane and heptachlor epoxide. Oven temperatures and flow rates

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³Mention of a company does not imply endorsement of its products by the U.S.D.A.

Table 1.—Properties of the soil at each location.

Location	pH	Readily oxidizable organic matter (%)	Sand (%)	Silt (%)	Clay (%)
Florida	4.40	0.36	94	3	3
Hawaii	5.80	18.85	66	23	11
Missouri	4.50	2.65	39	46	15
Oregon	7.15	.41	92	6	2
South Carolina	5.10	1.45	78	11	11

were greatly varied for both the polar DC-200 and the nonpolar SE-30 columns to change peak resolution. Qualitative comparisons were made with standards analyzed under the same conditions and with samples spiked with standards. Extraction *p*-values (Beroza and Bowman 1965a, b) were also determined for insecticides giving specific peaks by single distribution between 5-ml volumes of hexane and acetonitrile. The results were compared with those of standards partitioned with the same solvents and at the same temperature.

RESULTS AND DISCUSSION.—Initial penetrations of technical heptachlor emulsions in soils were determined when the tests were installed. The gas chromatograms were similar for all locations. They had a peak for gamma-chlordane (*cis*-isomer) in addition to the major heptachlor peak. Generally, with the proper dilution of the extract for analysis, only these 2 peaks were large enough to be calculated. When a more concentrated sample was injected, small peaks for minor components appeared. Usually 2 small peaks appeared after that of gamma-chlordane. Some of these minor components may be more persistent in certain soils than heptachlor or gamma-chlordane. Thus, they may appear as prominent compounds in residue studies in later years.

Chromatograms obtained for soil sampled after weathering for a year or longer varied considerably for the 5 locations. Table 2 summarizes the composition of the major insecticides in the soil samples taken 1, 2, and/or 3 years after application of technical heptachlor. The values shown are means from 3 plots. In most cases variations between replications were small, but for some soils a considerable spread in values was obtained. Large variations are to be expected in field studies, where distribution and penetration of insecticide are uneven. Differences in bulk density of the soils are also reflected in the results reported on a weight basis (ppm) for a specified layer of soil.

The chromatograms of Missouri soil extracts for both the 2nd and 3rd years were similar to those obtained from samples at the time of installation. In only a few cases were peaks corresponding to heptachlor epoxide large enough to be calculated. Although the values are lower for the 3rd year, the results indicate that both heptachlor and gamma-chlordane are relatively stable and persistent in the Lebanon silt loam soil.

The typical chromatogram of extracts from the Quincy loamy fine sand of Oregon depicted a diminished peak for heptachlor and a new peak corresponding to a standard of 1-hydroxy-chlordene. In

these samples, the values for 1-hydroxy-chlordene were relatively high. The new peak accounted for ca. 60% of the insecticide in the soil.

Both 1-hydroxy-chlordene and heptachlor epoxide were found in the Lakeland sand samples from Florida. Only a trace of the epoxide was present after 1 year, but significant amounts were obtained after 3 years. Although concentrations of 1-hydroxy-chlordene were considerably smaller than those in Oregon soil, significant amounts were obtained for all 3 years.

The prominent peaks in most chromatograms of the extracts of Cataula loamy sand from South Carolina were for heptachlor and gamma-chlordane. Small peaks for 1-hydroxy-chlordene were calculated for samples from 1 plot for the 1st year and for heptachlor epoxide from several plots for the 2nd and 3rd years.

In the 1st-year Makalapa clay samples from Hawaii only trace amounts of the degradation products were apparent. For the 2nd year, only samples from 1 plot were available for analysis of soil that had insecticide applied at 1 pint/ft², and the results were unexplainably low. In contrast, relatively high values were obtained for plots where 2 pints/ft² were applied. In several Hawaii plots, the peak for heptachlor epoxide was very large.

For comparisons, the data for the 2nd-year samples are presented as percent composition in Table 3. Since only the major insecticides in the extracts were considered in the calculations, the percentages are approximate and indicate trends only. In many cases the extract had to be greatly diluted to get the proper concentration for analysis of the major peaks. A more concentrated sample possibly would have given small peaks for 1-hydroxy-chlordene and heptachlor epoxide. We therefore do not say that no 1-hydroxy-chlordene and heptachlor epoxide exist at certain locations, but only that their concentrations are insignificant in comparison with the parent compound and gamma-chlordane. Percentages of 1-hydroxy-chlordene were negligible for the Missouri silt loam and the loamy sand of South Carolina, small for the Makalapa clay of Hawaii, generally small for Lakeland sand of Florida though high for several plots, and very high for the Quincy loamy fine sand of Oregon. Heptachlor epoxide usually represented a small fraction of the total insecticide present in the soils in the 2nd year. The highest percentages were found in Hawaii on the single plot to which 1 pint/ft² insecticide had been applied. On this plot concentrations of all insecticides were unusually low.

Whereas considerable attention in the literature has been given to the oxidation of heptachlor to heptachlor epoxide, very little information has been reported on the degradation of heptachlor to 1-hydroxy-chlordene. Fig. 1 shows structures of the 3 compounds. Bowman et al. (1965a) showed that heptachlor was readily changed to 1-hydroxy-chlordene when heptachlor-treated dry soils containing little organic matter were aged in a gravity-convection oven at 45°C for 4 days. Heptachlor was also found to be partially degraded to 1-hydroxy-chlordene during the preparation of an insecticidal dust used for soil treatment in a study on control of a white-fringed beetle, *Graphognathus perigrinus* (Buchanan) (Bowman et al. 1965b). Further degradation was not detected after the dust was added to

Table 2.—Insecticide residues (ppm) found at various depths in soil 1, 2, and/or 3 years after application of water emulsions of 0.5% technical heptachlor at 1 and 2 pints ft².

State	Year	Layer (inches)	1 pint ft ²				2 pints ft ²			
			H ^a	GC ^b	HC ^c	HE ^d	H	GC	HC	HE
Florida	1	0-1	148.	90.9	16.7	tr*	289.	149.	30.8	tr
		1-2	67.3	31.7	11.2	tr	424.	123.	34.8	tr
		2-4	15.0	8.2	5.4	tr	102.	32.0	14.3	tr
	2	0-1	210.	71.3	11.5	0.9	119.	56.7	17.5	1.6
		1-2	39.5	18.7	5.1	.7	186.	44.7	14.8	0.7
		2-4	14.7	7.6	3.9	1.4	101.	26.7	10.1	.6
	3	4-6	4.0	1.5	1.7	.3	54.6	11.0	7.4	tr
		0-1	135.	72.1	9.7	3.2	64.3	75.9	10.7	11.8
		1-2	57.8	30.0	6.2	3.9	146.	56.5	6.6	4.2
		2-4	20.0	13.3	2.9	3.3	99.0	32.8	4.6	3.7
		4-6	6.0	4.1	1.4	1.7	15.4	9.4	2.2	1.9
Hawaii	1	0-1	350.	155.	tr	tr	292.	108.	tr	tr
		1-2	318.	96.0	tr	tr	156.	51.6	tr	tr
		2-4	102.	35.4	tr	tr	110.	35.2	tr	tr
	2	0-1	14.6	14.9	1.7	10.5	567.	262.	56.5	37.7
		1-2	12.0	7.4	1.5	7.5	716.	230.	40.5	8.8
		2-4	2.1	0.5	tr	1.5	303.	106.	18.4	10.9
Missouri	2	0-1	688.	210.	— ^e	—	840.	262.	—	—
		1-2	421.	119.	—	—	629.	185.	—	—
		2-4	146.	42.0	—	—	207.	79.6	—	—
	3	0-1	431.	151.	—	5.5	634.	215.	—	—
		1-2	68.7	25.7	—	3.9	210.	83.3	—	—
		2-4	30.5	11.8	—	2.5	159.	50.6	—	—
Oregon	1	0-1	35.0	130.	230.	—	46.6	195.	413.	—
		1-2	11.1	11.9	32.8	—	9.7	22.1	63.5	—
		2-4	0.9	.9	2.6	—	1.1	2.0	5.3	—
	2	0-1	55.4	121.	237.	—	132.	197.	380.	—
		1-2	7.2	9.4	29.4	—	29.2	39.2	95.0	—
		2-4	1.1	1.1	3.7	—	4.4	5.5	14.7	—
South Carolina	1	0-1	313.	103.	5.9	—	588.	189.	tr	—
		1-2	141.	36.6	2.9	—	312.	83.5	tr	—
		2-4	10.8	5.6	1.4	—	166.	26.4	tr	—
	2	0-1	530.	127.	—	—	841.	207.	—	—
		1-2	123.	39.5	—	2.0	208.	55.4	—	—
		2-3	8.8	4.2	—	.9	138.	38.3	—	—
	3	3-4	8.0	2.3	—	tr	10.4	3.3	—	—
		0-1	359.	131.	—	1.6	457.	167.	—	—
		1-2	133.	40.8	—	1.9	196.	59.7	—	—
		2-4	6.6	3.9	—	1.4	24.2	10.3	—	1.3

^a Heptachlor.^b Gamma-chlordane.^c 1-Hydroxy-chlordene.^d Heptachlor epoxide.^e Trace.^f Peak either missing or too small to calculate.

the soil. In the bioassay system, 1-hydroxy-chlordene was essentially nontoxic and did not synergize heptachlor and or gamma-chlordane. The presence of 1-hydroxy-chlordene in a small number of soil samples collected in the Atlantic Provinces of Canada was reported by Dutt and Wong (1967). Beverue and Yeo (1969a, b) found that within 30 days of exposure to water, the heptachlor component of chlor-

dane was completely changed to 1-hydroxy-chlordene.

The data thus far obtained in our studies have shown that the fate of heptachlor varies from one application to another. At this time we do not know which factors are responsible for the variations. Additional results from this long-term field experiment as well as information obtained from studies recently established should aid in explaining the differences. Additional field experiments will be set up in 1970 at the 5 locations to amplify the information presently known. Volatilization, adsorption on soil particles, leaching, chemical alteration, photodecomposition, and microbial degradation all appear to be important processes in the removal or inactivation of insecticides, and consequently in the determination of their long-term effectiveness (Bowman et al. 1965a, Edwards 1961). The biological activity of an insecticide in soil thus depends not only upon the nature

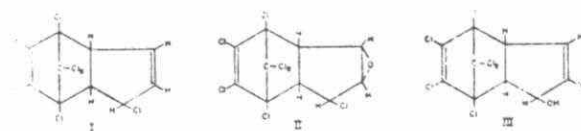


FIG. 1.—Structures for heptachlor (I), heptachlor epoxide (II), and 1-hydroxy-chlordene (III).

Table 3.—Relative amounts of major insecticides (% composition) found at various depths in soil 2 years after application of water emulsions of 0.5% technical heptachlor at 1 and 2 pints/ft².

State	Layer (inches)	1 pint/ft ²				2 pints/ft ²			
		H ^a	GC ^b	HC ^c	HE ^d	H	GC	HC	HE
Florida	0-1	71.5	24.3	3.9	0.3	61.1	29.1	9.0	0.8
	1-2	61.7	29.2	8.0	1.1	75.5	18.2	6.0	0.3
	2-4	53.3	27.5	14.1	5.1	73.0	19.3	7.3	0.4
	4-6	50.0	18.8	21.3	10.0	74.8	15.1	10.1	0.0
Hawaii	0-1	35.0	35.7	4.1	25.2	61.4	28.4	6.1	4.1
	1-2	42.2	26.1	5.3	26.4	71.9	23.1	4.1	0.9
	2-4	51.2	12.2	—	36.6	69.1	24.2	4.2	2.5
Missouri	0-1	76.6	23.4	—	—	76.2	23.8	—	—
	1-2	78.0	22.0	—	—	77.3	22.7	—	—
	2-4	77.7	22.3	—	—	78.9	21.1	—	—
Oregon	0-1	13.4	29.3	57.3	—	18.6	27.8	53.6	—
	1-2	15.7	20.4	63.9	—	17.9	24.0	58.1	—
	2-4	18.6	18.6	62.7	—	17.9	22.4	59.8	—
South Carolina	0-1	80.7	19.3	—	—	80.2	19.8	—	—
	1-2	74.8	24.0	—	1.2	79.0	21.0	—	—
	2-3	63.3	30.2	—	6.5	78.3	21.7	—	—
	3-4	77.7	22.3	—	—	75.9	24.1	—	—

^a Heptachlor.^b Gamma-chlordane.^c 1-hydroxy-chlordane.^d Heptachlor epoxide.

and quantity of the compound, but also upon soil properties and environmental conditions (Harris 1966).

In a preliminary laboratory test of heptachlor, heptachlor epoxide, gamma-chlordane and 1-hydroxy-chlordane against the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), the first 2 compounds appeared to be more toxic than gamma-chlordane which, in turn, was more toxic than 1-hydroxy-chlordane. Since the insecticidal properties of the 4 chemicals vary considerably, the degradation of heptachlor to heptachlor epoxide and 1-hydroxy-chlordane may prove important in the effective control of subterranean termites in different soils and climates in the United States. Because the field tests have been too recently installed to evaluate the effect of such degradation in the various soils, annual sampling will be continued to monitor any conversion and to assess its effect on termite control.

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1-Hydroxy-2,3-Epoxy-chlordene in Oregon Soil Previously Treated with Technical Heptachlor

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Gas-chromatographic analyses of residues and degradation products of technical heptachlor weathered 4 years in an Oregon soil revealed an unknown component in layers below the soil surface. The degradation product was either missing or minor in the surface samples and in all samples taken after 1 or 2 years of weathering. In addition, it was not apparent on chromatograms of extracts of four other soils in test. Because the gas-chromatographic peak attributed to the unknown was larger than the peaks of residual heptachlor and gamma-chlordane, the work described in this paper was undertaken to isolate and identify the unknown component.

Methods and Materials

Soil samples were taken from test plots established to determine initial penetration of the soil by technical heptachlor at five field locations. The installation, locations, soil properties, and analytical methods have been previously reported (1,2).

The soil extracts and their fractions were analyzed with several gas chromatographs with different columns and operated under various conditions. Qualitative comparisons were made with standards analyzed under the same conditions and with samples spiked with standards. Column packings included 3% DC-200 on 100/120 mesh Gas Chrom Q,^{1/} 3% SE-30 on 60/70 mesh Chromport XXX, 11% (QF-1 plus OV-17) by weight on 80/100 mesh Gas Chrom Q, and 9% QF-1 on 100/120 mesh Gas Chrom Q.

Extraction *p*-values (3,4) were determined for insecticides giving specific peaks by single distribution between 5-ml volumes of hexane or pentane and acetonitrile. The results were compared with those of standards partitioned with the same solvents and at the same temperature.

^{1/} Mention of a company or trade name does not imply endorsement by the U.S. Department of Agriculture.

The major components in certain Oregon soil extracts were fractionated on columns of silica gel (powder, suitable for chromatograph use, Baker Analyzed Reagent). The solvents were either hexane followed by mixtures of hexane and isopropyl alcohol or pentane followed by mixtures of pentane and diethyl ether.

Mass spectra were obtained on a Consolidated Electrodynamics Corporation Mass Spectrometer, Model 21-110B, using the direct introduction system. Peaks were identified by the use of perfluorokerosene as a reference.

Results and Discussion

An unknown peak, not appearing on the chromatograms of soil extracts from other locations, occurred in the chromatograms from the Oregon soil extracts. The peak was most prominent in chromatograms of extracts of soils sampled at the 2- to 4-inch layers. The exact amount could not be determined, as a standard was not available at the time of analysis. The retention time of the unknown component was only slightly more than that of heptachlor epoxide on the 3% DC-200 column. However, as a result of its tailing, the unknown peak was more poorly resolved from the gamma-chlordane peak than was a standard heptachlor epoxide peak.

Gas chromatograms of an extract of the Oregon soil sampled 1 to 2 inches below the surface have eight peaks (Fig. 1A,B). Peaks 2, 4, 6, 7, and 8 were identified tentatively as heptachlor, 1-hydroxychlordane, gamma-chlordane, alpha-chlordane, and nonachlor. Peaks 1 and 3 (shoulder on peak 4) were small, but the fifth peak was larger than the residual heptachlor and gamma-chlordane peaks and therefore was considered to be significant enough to warrant isolation and identification.

A 100-ml aliquot of the extract (hexane) was fractionated on a 10-g silica gel column (15 mm I.D.). Components corresponding to peaks 1, 2, 6, 7, and 8 were either not retained on the column or readily eluted from it with 100 ml pentane (Fig. 1C). Additional pentane (300 ml) eluted a component with a retention time on the 3% DC-200 column slightly less than that of peak 5 and corresponding to heptachlor epoxide (Fig. 1D). Mixtures of pentane and diethyl ether eluted the more polar components corresponding to peaks 3, 4 (1-hydroxychlordane), and 5 (Fig. 1E,F). Although the heptachlor epoxide peak was overlapped and hidden by peak 5 in the chromatograms of the unfractionated soil extract (Fig. 1A,B), the amount of heptachlor epoxide present in the extract was quite small compared with that of the unknown. The two components were separated on the 11% (QF-1 plus OV-17) column, but then the unknown and gamma-chlordane peaks were not resolved.

For isolation of larger quantities of the components, additional extracts were prepared of the Oregon soil remaining from previous analyses. By rechromatographing fractions on silica gel columns and varying the solvents and amount of silica gel,

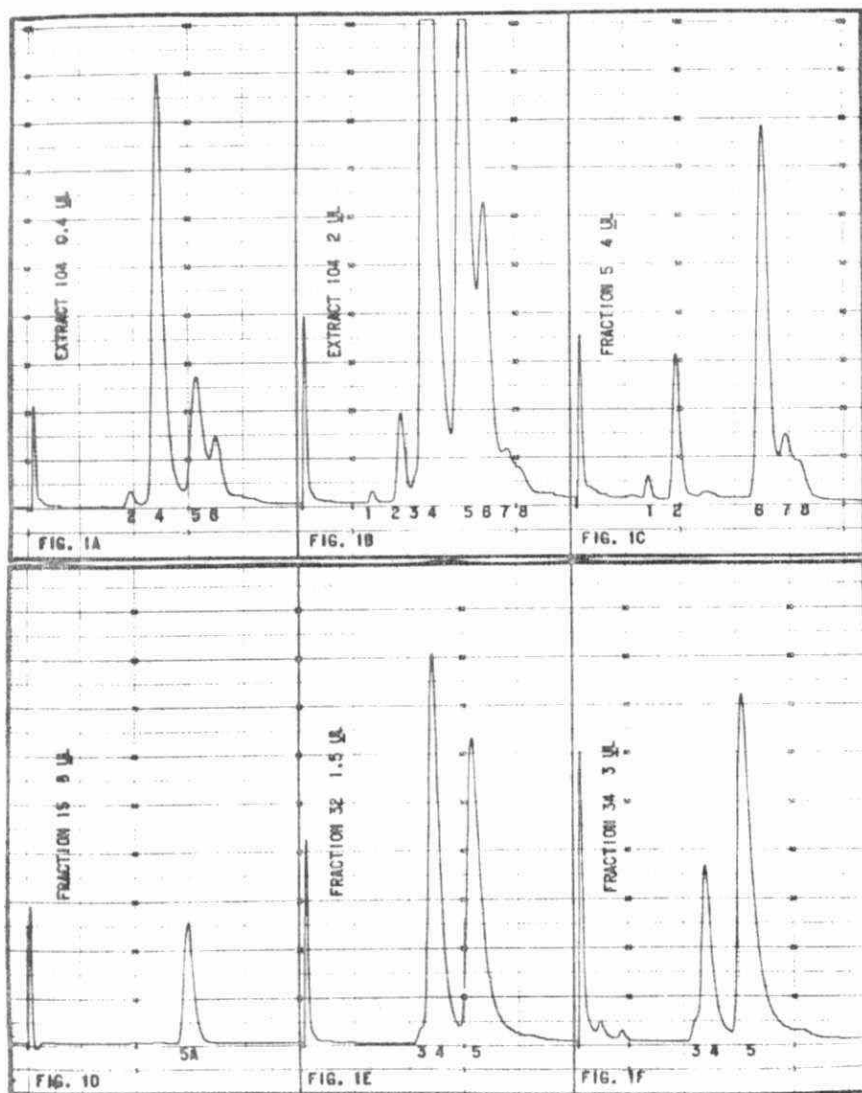


Figure 1. Gas chromatograms (3% DC-200 column) of Oregon soil extracts and fractions of the extract through a silica gel column: (A) 0.4 μ l of original extract; (B) 2.0 μ l of original extract to show minor peaks; (C) fraction 5, eluted with pentane; (D) fraction 15, eluted with pentane; (E) fraction 32, eluted with 90:10 pentane:diethyl ether; (F) fraction 34, eluted with 85:15 pentane:diethyl ether.

fractions consisting mainly of one component were obtained. Hexane and isopropyl alcohol which were used in preliminary experiments were replaced by pentane and diethyl ether in later experiments. The silica-gel column fractions were analyzed on several columns in different gas chromatographs. In all cases, peaks 2, 4, 5A, 6, 7, and 8 corresponded to standards of heptachlor, 1-hydroxychlordene, heptachlor epoxide, gamma-chlordane, alpha-chlordane, and nonachlor.

Previously undetected components became apparent in certain fractions. Although these components could be breakdown products of the other components on the silica gel column, we believe they are minor components which were concentrated by being retained on the column and then eluted in specific patterns. Although in many cases a very large and sharp peak would be obtained for such components, the entire quantity of the specific component was too small for identification. Fractions that contained unusual components are being saved for possible reference materials for later studies.

The unknown component (peak 5) was postulated to be a degradation product of 1-hydroxychlordene, the major component in the Oregon soil after weathering for only 1 year. The elution of both the unknown and 1-hydroxychlordene from the silica gel column was similar and gas chromatographic peaks of both tended to tail. In a recent paper, Miles, Tu, and Harris (5) reported the epoxidation of 1-hydroxychlordene by soil microorganisms to 1-hydroxy-2,3-epoxychlordene. This hydroxy-epoxide has also been reported by Kaul *et al.* (6) as a metabolite in rats which were intravenously injected with heptachlor, and by Brooks and Harrison (7) as a metabolite of chlordene in the housefly, *Musca domestica* L. Brooks (8) reported that both 1-hydroxychlordene and 1-hydroxy-2,3-epoxychlordene were nontoxic when topically applied to adult female houseflies.

The retention time of the unknown on four gas chromatographic columns agreed with those of an authentic reference standard of 1-hydroxy-2,3-epoxychlordene furnished by Dr. Percy B. Polen of Velsicol Chemical Corporation. The *p*-values (3,4) obtained with pentane and acetonitrile also checked. Mass spectra were compared for the standard hydroxy-epoxide and the unknown. The parent peak end of the spectra of both known and unknown showed a group of peaks of similar relative intensities. The lowest of these had a *m/e* value of 368, corresponding to the formula $C_{10}H_6O_2Cl_6^{35}$. Higher *m/e* values corresponded to the various $Cl^{35}-Cl^{37}$ combinations, with the *m/e* for $-Cl_6^{37}$ (380) being barely apparent. The data thus indicate that the component found in the Oregon soil which had been treated 4 years previously with technical heptachlor is 1-hydroxy-2,3-epoxychlordene.

For all soils except the Quincy loamy fine sand of Oregon, heptachlor and gamma-chlordane (trans-isomer present in technical heptachlor) are still the major components (Table I). Essentially no heptachlor remained in the Oregon soil, and the major component was 1-hydroxychlordene. This degradation product of heptachlor was also found in the Hawaii (Makalapa clay) and Florida (Lakeland sand) soils. Relatively high values for heptachlor epoxide were found for the Hawaii and Missouri (Lebanon silt loam) soils. Only traces of 1-hydroxychlordene and heptachlor epoxide were detected in the soil from South Carolina (Cataula loamy sand).

TABLE I

Major insecticide residues (ppm) found in upper 1-inch layer of various soils weathered for 4 years after application of water emulsion of 0.5% technical heptachlor at 2 pt/sq ft

Field location	Heptachlor	γ -chlordane	1-OH-chlordene	Heptachlor epoxide
Florida	148.	90.1	13.6	1.9
Hawaii	425.	387.	41.0	95.9
Missouri	472.	191.	...*	29.5
Oregon	0.0	106.	238.	...
South Carolina	482.	142.

* Peak either missing or too small to calculate.

Research reported^{2/} here is part of a field evaluation of chlorinated hydrocarbons^{2/} for control of various species of termites in different soils and climates in the United States (1). After 1 year at the Oregon test site, much of the heptachlor was converted to 1-hydroxychlordene, the major component in the extracts prepared from the soil (2). After 4 years essentially no heptachlor remained, and a portion of the 1-hydroxychlordene had further degraded to 1-hydroxy-2,3-epoxychlordene. Thus, under certain conditions such as these found at the Oregon test site, a major pathway of heptachlor degradation in soil is hydrolysis to 1-hydroxychlordene, which then can be epoxidized. Epoxidation was probably effected by soil microorganisms (5). To our knowledge, 1-hydroxy-2,3-epoxychlordene has not been reported previously as a degradation product in heptachlor-treated soils.

^{2/} This publication reports research involving pesticides. It does not contain recommendations for their use nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

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EFFECTS OF REPEATED APPLICATIONS OF PESTICIDES TO SOIL¹

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ABSTRACT

The effects of repeated applications of various pesticides to the soil were investigated during 1949 to 1953 in a field experiment at Kentville, N.S. Applications of arsenic, DDT, ferbam, and sulphur caused a decrease in the yields of some crops. DDT, arsenic, and BHC accumulated in the soil, whereas parathion and sulphur did not. There was evidence of translocation of parathion and BHC in plants. Arsenic, sulphur, ferbam, and BHC influenced the chemical composition of the soil.

INTRODUCTION

The introduction of various types of organic compounds has greatly increased the number and variety of pesticides in general use. Many of the newer materials, as well as others that have been in use for a considerable time, are highly toxic. This and the possibility of accumulations resulting from repeated yearly applications make it essential to have information on the effects of pesticide residues in soils.

In the spring of 1949, a field experiment involving pesticides commonly used in orchard sprays was initiated at Kentville, N.S. The results obtained during a 5-year period are presented in this paper.

MATERIALS AND METHODS

The experimental layout was a randomized block design with four replications. The individual plots were 13 ft. × 16 ft. (approximately 1/200 acre) and were separated by 5-foot cultivated strips. The soil in the experimental area has been classified as Berwick sandy loam (9), which is one of the common soil types in the Annapolis Valley.

The pesticides were applied annually in late May or early June before planting. They were added to the surface of the plots in water suspension and then thoroughly incorporated into the soil to a depth of approximately 6 inches with a rotary cultivator. Treatments, together with the rates and years of application, are given in Table 1.

It was estimated that the amounts of pesticides applied annually were roughly comparable to those that would be used in an average spray program during a 5-year period, and thus information in regard to the possible effects of long-term spray programs would be obtained in a relatively short time. Taking an acre of soil to a depth of 6 inches as weighing 2,000,000 lb., the concentration of active ingredient resulting from each application would be: arsenic (As), 45 p.p.m.; DDT, 52.3 p.p.m.; parathion, 15.7 p.p.m.; sulphur (S), 628 p.p.m.; ferbam, 73.2 p.p.m.; BHC 13 p.p.m.; and chlordane, 5 p.p.m.

¹ Contribution No. 282, Chemistry Division, and No. 3318, Entomology Division, Science Service, Canada Department of Agriculture, Ottawa.

TABLE 1.—SOIL TREATMENTS

Pesticide	Annual application (lb./ac.)	Years of application
Lead arsenate (PbHAsO_4)	419	1949, 1950, 1951, 1952, 1953
DDT (50% W.P.)	209	1949, 1950, 1951, 1952, 1953
Parathion (15% W.P.)	209	1949, 1950, 1951, 1952, 1953
Sulphur (S)	1,256	1949, 1950, —, —, —
Ferbam (70% active ingredient)	209	1949, 1950, 1951, 1952, 1953
BHC (50% W.P. 6% gamma)	52	—, 1950, 1951, 1952, 1953
Chlordane (40% W.P.)	25	—, —, 1951, 1952, 1953

Beans, buckwheat, carrots, and potatoes were used as test crops from 1949 to 1953, inclusive. Onions were included in 1951 and 1952. Either 3-15-6 or a 6-12-6 fertilizer at 800 or 1,000 lb. per acre was applied annually and manure was also used in 1949 and 1951. The crops were grown in the same relative position each year but were rotated annually within each plot so that the same crop was not grown in the same row in successive years. At maturity the crops were harvested, yields recorded, and representative samples taken for the determination of pesticide content.

Composite soil samples, representative of the 0-6 inch depth, were taken from each plot before and immediately after each annual pesticide application and again at the end of the growing season. Determinations for nitrate nitrogen were made according to the A.O.A.C. method (11) and organic matter, exchangeable bases (calcium, magnesium, potassium), total and available phosphorus, exchangeable manganese, and pH values were determined by methods given by Wright *et al.* (14).

The following methods were utilized in determining the pesticide content of soil and plant samples: arsenic, Allcroft and Green (2) and A.O.A.C. (11); DDT, Donovan (7) and Schechter *et al.* (13); parathion, Averell and Norris (4); BHC, Donovan (7) and Schechter and Hornstein (12); chlordane, Davidow (6); sulphur, soil samples extracted with carbon tetrachloride, plant samples wet ashed with nitric and perchloric acids, sulphates precipitated with barium chloride and weighed. A satisfactory chemical method for the determination of ferbam was not available.

Crop yield data as well as the results of chemical analyses were treated statistically by making an analysis of variance, and Student's *t* test was used to determine what treatments were significantly different from the check.

RESULTS AND DISCUSSION

Crop Yields

The average yields are presented in Table 2.

Arsenic, applied to the soil as PbHAsO_4 , was considered the toxic element in the lead arsenate, although the possibility of lead toxicity is recognized. In plots treated with lead arsenate there was a significant reduction in the yield of beans in 1950 and in each succeeding year up to and including 1953; no consistent effects on the yields of other crops

TABLE 2.—EFFECTS OF PESTICIDES ON CROP YIELDS
(Average yields of four replicates—pounds per plot)

Crop	Year	Pesticides applied							Check
		Arsenic	DDT	Parathion	Sulphur	Ferbam	BHC	Chlordane	
Potatoes (tubers)	1949	44.3	46.1	40.7	37.0	38.1	—	—	38.5
	1950	16.1*	20.0	23.9**	2.8**	16.2*	25.9**	—	19.2
	1951	19.4	21.6	17.1	6.0**	15.4	19.2	22.3	19.1
	1952	15.4	19.4	21.1	0.8**	15.5	20.6	18.5	16.1
	1953	18.6	26.0	29.3*	16.2	23.4	25.5	25.3	20.9
Carrots (roots)	1949	24.4	28.0	25.1	12.4**	23.6	—	—	25.5
	1950	18.7	15.9	11.6	0.0**	13.1	12.8	—	15.4
	1951	25.3	24.4	19.7	2.2**	16.1	15.2	20.3	19.5
	1952	10.0	12.8	10.9	0.0**	7.5	17.2	9.6	13.7
	1953	31.6	42.3	34.3	5.1**	23.5**	38.8	36.6	35.8
Beans (green beans)	1949	3.2	3.1	3.8	2.5**	3.1	—	—	3.4
	1950	3.1**	2.7**	4.2	0.3**	3.4**	4.9	—	4.7
	1951	2.4**	1.9**	5.4	2.2**	3.3**	5.8	5.8	6.1
	1952	2.8**	2.9**	6.4*	1.7**	5.2**	8.0	6.5	7.9
	1953	1.2**	1.1**	5.6	2.2**	3.7	5.4	4.5	4.8
Buckwheat (aerial portion)	1949	19.4	18.4	20.4	7.9**	17.3*	—	—	21.7
	1950	17.8*	12.9	16.4	2.5**	6.1**	14.6	—	12.5
	1951	19.8**	13.2	13.5	11.5	12.1	11.9	15.8	14.8
	1952	22.4	14.4	20.0	16.6	16.8	15.4	18.0	21.7
	1953	16.7	8.7**	12.3	14.9	11.6	10.4*	13.0	15.9
Onions (bulbs)	1951	8.4	5.9*	7.0	0.1**	3.4**	5.7*	6.5	8.3
	1952	4.9	2.2*	3.9	0.2**	1.4**	2.6	3.3	3.6

* Significant at P 0.05.

** Significant at P 0.01.

resulted. Serious accumulations of arsenic have been reported where heavy dosages have been applied for insect control. Foster (8), reviewing the arsenical residue problem in the western United States, reported that arsenic accumulations in the surface soil were largely responsible for increasing difficulties in the growing of certain cover crops although deep-rooted orchard trees remained unharmed. Cooper *et al.* (5) reported a marked reduction in certain crops grown on areas previously sprayed with arsenicals for control of cotton insects.

DDT caused a significant reduction in the yield of beans in the years 1950 to 1953, inclusive, of buckwheat in 1953, and of onions in 1951 and 1952. Several workers have reported reduction of crop yields resulting from heavy applications of DDT to the soil. Lindgren *et al.* (10) have shown that 128 lb. or over of DDT per acre caused reduction of yield of Stringless Black Valentine bush beans. A report by Foster (8) states that 100 lb. per acre caused a reduction in the yield of the same variety of beans. Ackley *et al.* (1) have reported that residues of DDT resulting from treatments for insect control had approached phytotoxic levels when soil samples in 29 orchards of the Wenatchee and Yakima areas showed an accumulation of 22 to 62 lb. of DDT per acre in the surface 4 inches of soil under the spread of the trees.

Parathion-treated plots showed no consistent differences from the checks in crop yields. It appears improbable that phytotoxic accumulations of parathion will result from pest control treatments at recommended dosages. Foster (8) states that toxic residues in the soil are not an object of concern but indicates that a temporary depressing effect on germination and stand may occur.

Sulphur, which was applied only in 1949 and 1950, caused reduction in crops yields of carrots and beans in 1949 to 1953, inclusive, of potatoes in 1950 to 1952, inclusive, of buckwheat in 1949 and 1950, and of onions in 1951 and 1952. There was, therefore, a yield recovery of buckwheat in one year and of potatoes in three years after the last sulphur treatment.

Ferbam reduced the yield of beans in 1950, 1951, and 1952, of potatoes in 1950, of carrots in 1953, of buckwheat in 1949 and 1950, and of onions in 1951 and 1952. The effects of this material, which were seemingly inconsistent, suggest the possibility of indirect or secondary effects on crop growth.

BIIC caused a highly significant increase in the potato crop in 1950, the first year this pesticide was applied. A significant decrease in the yield of onions resulted in 1951 but did not recur the following year. The buckwheat crop was decreased in 1953. The rather inconsistent results indicate a secondary or indirect rather than a straight toxic effect on the crops. Foster (8) found that technical BHC at 400 lb. per acre "virtually eliminated all growth" of some 15 crops being tested. The highly purified gamma isomer of BHC, however, is reported by the same workers as having no adverse effects on germination and stand of crops even at concentrations of 400 lb. per acre.

Chlordane treatments applied in 1951, 1952, and 1953 at the rate of 10 lb. actual toxicant per acre did not affect the yield of the test crops. Foster (8) found that chlordane at 25 lb. per acre depressed the stand of most vegetable crops.

Accumulation of Pesticides in the Soil

The concentrations of the active ingredients of the added pesticides at dates of application and at the end of each growing season for the years 1949 to 1953, inclusive, are presented graphically in Figure 1.

Arsenic, *DDT*, *BIIC*, and *chlordane* appeared to be relatively stable in the soil. Although there was a gradual decrease in concentration of these compounds in the soil throughout the year, their repeated application resulted in a build-up. Allen *et al.* (3) reported that plots treated with 100 lb. of DDT per acre in 1947 had a residue of 28.2 lb. per acre in 1951 in the top 6 inches of soil, and that after 3 years about one-half of a 100 lb. per acre application of BHC had disappeared.

Parathion disappeared rapidly from the soil. After annual spring applications of 15.7 p.p.m. active ingredient over a period of 5 years, the final concentration in the soil at the end of the fifth year was only 0.5 p.p.m. This finding agrees with the conclusions of Foster (8) on the instability of the compound.

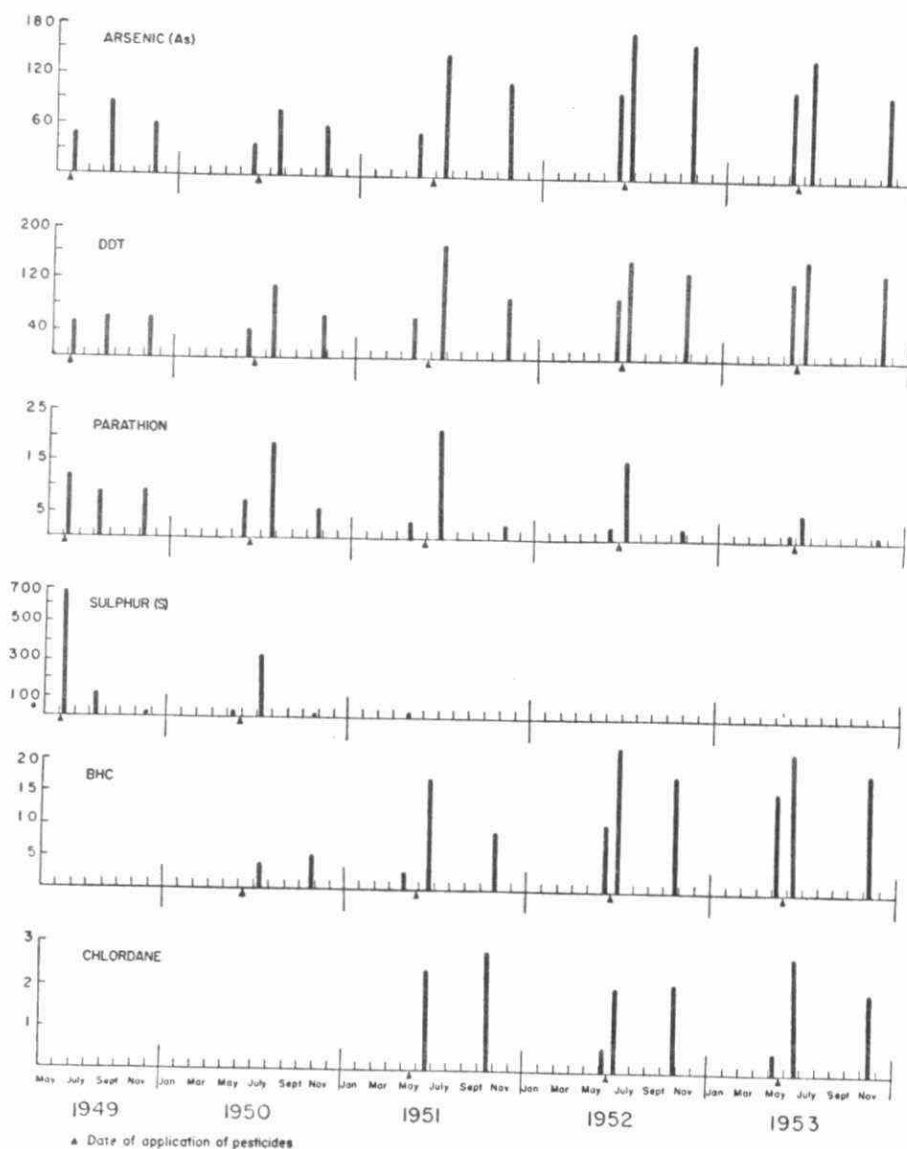


FIGURE 1. Concentration of pesticides in the soil in p.p.m. of active ingredient, air dry basis.

Sulphur applications resulted in marked increases in soil acidity, and elemental sulphur rapidly disappeared.

Translocation of Pesticides to Plants

Chemical analyses were made of the plants grown and the following conclusions arrived at as to translocation.

There was definite evidence of translocation of parathion in beans and buckwheat. Although parathion was not found in the edible part of the bean plant, that is, the pod and contents, it was found in the leaves and stems. It was found also on the surface of carrots and on the peel of potato tubers grown in parathion-treated soil.

There did not appear to be any appreciable degree of translocation of DDT or chlordane in the plants grown in this experiment. Traces were found only on the surface of carrots and potatoes, and were probably due to contamination from the soil.

Translocation of BHC to root crops, that is, potatoes, carrots, and onions, was indicated by organoleptic tests and the presence of BHC was definitely shown by microchemical analytical methods.

Increased concentrations of arsenic in the soil resulted in an increased arsenic content in the plants.

Crops tended to avail themselves of increased sulphur in the soil provided the sulphur concentrations were not such as to prove injurious to crop development.

Effects of Pesticides on the Chemical Composition of Soil

Soil fertility investigations by chemical analytical methods indicated the following:

Arsenic, considered to be the active ingredient in lead arsenate, had no significant effect on pH, readily soluble phosphorus (Truog's method), exchangeable basis, exchangeable manganese, or organic matter. Nitrate nitrogen content was significantly higher in the 5-year averages but not on a yearly basis.

DDT, parathion, and chlordane had no significant effect on the pH of the soil or on the concentrations of the plant nutrients determined.

Sulphur added as elemental sulphur at 1,256 lb. per acre in each of two successive years lowered the pH of the soil from 5.16 in 1949 to 3.70 in 1950. Although sulphur was not added in the last three years of the experiment, in 1953 the pH of the treated plots was still significantly lower than that of the checks. There was, however, a gradual decrease in soil acidity from 1951 to 1953. Nitrate nitrogen content was significantly lowered. The concentrations of the exchangeable bases calcium and magnesium were significantly decreased as a result of the sulphur treatments but the 5-year average for potassium was found to be not significantly different from that for the check plots.

Ferbam significantly decreased the nitrate nitrogen content of the soil in the third, fourth, and fifth years of the experiment and the 5-year average was also significantly reduced. The amount of exchangeable manganese was significantly increased in the third and fifth years as was also the 5-year average.

BHC treatments significantly increased the exchangeable manganese content in each of the four years it was applied.

CONCLUSIONS

The continuous use of arsenic, DDT, ferbam, or sulphur for pest control purposes presents the danger of an eventual build-up in the soil that may be detrimental to plant growth. This is particularly true where comparatively large dosages are applied repeatedly, as in orchards.

The effect of ferbam on the nitrate nitrogen content and that of BHC on exchangeable manganese indicated that these compounds exerted an influence on soil organisms.

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Residues in Soybeans Grown in Soils Treated with Heptachlor or Chlordane Or Sprayed with Chlordane

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THE USE OF HEPTACHLOR AND CHLORDANE to control insect pests of soybeans not only depends on their effectiveness as control agents but also on the magnitude and toxicological significance of resulting residues. Both of these toxicants are well established insecticides and have proven effective against a wide range of pests, especially soil insects. Thus, from the control standpoint, it is likely that they would be favorably considered for use in a soybean production program. Residue problems, if any, which might occur as a result of their use in this manner have not been well defined.

As with most insecticides, there was a reasonable expectation that residues at some level would be present in soybeans treated with heptachlor or chlordane. It is possible for the materials to be translocated from soil applications into plant parts used as food or feed stuffs. Both heptachlor and chlordane applied as granules to the soil resulted in relatively high residues in the meat of the peanut (Morgan et al, 1967). These authors concluded that applying 2 pounds of heptachlor per acre would cause residues of heptachlor epoxide in the soil at levels which would produce measurable residues in the next season's crop. In the case of wheat, heptachlor and heptachlor epoxide were translocated into the plant in sufficient quantity to kill larvae of the wheat stem sawfly in the stem (Wallace and Butler, 1967).

Similar reports have been presented in which soybeans were used as test plants. Eden and Arthur (1965) showed that soybeans grown on heptachlor-treated soil contained heptachlor and heptachlor epoxide residues. Translocation of the materials was not definitely estab-

lished since statistical analysis revealed that none of the residues was significantly different from the untreated check. A subsequent study (Bruce and Decker, 1966) showed that residues of heptachlor in soybeans were related to the concentration of pesticide in the soil. Residues in the whole soybeans resulting from treatment rates of 2, 5, 10 and 20 pounds of heptachlor per acre were 0.06, 0.12, 0.20 and 0.31 ppm, respectively. Ninety percent of the residues were in the form of heptachlor epoxide. These data were calculated on a whole-bean basis with the oil content being approximately 18 percent.

In addition to the importance of insecticide residues in products consumed directly by humans, one must be concerned with residue levels of heptachlor and chlordane that could cause indirect contamination of food products. This was exemplified by the work of Westlake, et al (1963). These workers showed that dairy cows grazing on pasture grass treated with chlordane produced milk containing residues of heptachlor epoxide and chlordane. Although the total residue level was low, less than 0.1 ppm, it occurred as a result of the cows feeding on grass containing 1.5 ppm or less chlordane residues.

Our current research supports the earlier findings that heptachlor and chlordane are translocated into plants, specifically soybeans, and points out the levels of residues found in soybeans grown locally in soils treated with these two insecticides. Additionally, similar studies were conducted following foliar application of chlordane to soybean plants.

METHODS AND MATERIALS

Four field plots, Lufkin Fine Sandy Loam, each 10 feet wide and 31.1 feet long, were used for each insecticide treatment. In tests designed to determine the quantities of residues in soybeans grown in hepta-

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clor- and chlordane-treated soils, the insecticides were applied to freshly disked plots in sufficient water to give a total of 9.6 gallons of spray per acre. Heptachlor was applied at rates of 3 and 6 pounds of actual toxicant per acre and chlordane was applied at rates of 8 and 12 pounds of actual toxicant per acre. The soil was disked following application of each insecticide, and 8 days later, May 5, 1967, the soybeans (Lee Variety) were planted. There were two rows planted in each field plot. Additional plots of soybeans were planted at the same time for use in tests involving the foliar treatment of soybeans with chlordane. Soybeans used for control samples were planted adjacent to the treated plots.

Approximately 3 months after planting, August 9, 1967, the soybeans grown in the untreated soils were sprayed with chlordane. Four plots were treated at a rate of 1 pound per acre, and another four plots were treated with 3 pounds of chlordane per acre. The total volume of spray was 3.5 gallons per acre. Seven days after the first foliar application, the same plots were sprayed again in an identical manner.

Soil samples were taken from all plots on the day the soybeans were planted. Twenty cores, 1 x 6 inches, were collected from each plot. Soybean plants were taken for residue analysis on September 21, 1967. At this stage of development, small bean pods were present on the plants. On October 25, the mature soybean seeds were harvested, shelled and stored in a sealed container. All samples were kept frozen until analyzed.

Rainfall during the interim of these tests was May 3, 5 inches; June 1, trace; July 9, 3 inches; August 2, 1 inch; September 9, 3 inches; and October 1, 2 inches. The plots were surface irrigated between rows on July 31 and August 2, 9 and 11.

Quantitation of heptachlor and chlordane residue was accomplished using a Barber-Colman Series 5000 gas chromatograph equipped with an electron capture detector. Samples taken from plots treated with heptachlor were analyzed for heptachlor, heptachlor epoxide and alpha-chlordane. Those taken from chlordane-treated plots were analyzed for gamma-chlordane, alpha-chlordane, heptachlor and heptachlor epoxide. Analytical standards of these materials were provided by the Velsicol Chemical Company. The use of a 6-foot-column containing 10 percent SE-30 Anakrom ABS, 80/90 mesh, operated at 175°C. gave retention times of 7.7, 13.2, 15.2 and 17.3 minutes for heptachlor, heptachlor epoxide, gamma-chlordane and alpha-chlordane, respectively.

Soil samples, 100 grams, were extracted with 200 milliliters of a 1:1 benzene-acetone mixture. The extract was washed with water, and the organic solvent layer was dried with anhydrous sodium sulfate. The extract

was concentrated and analyzed without further cleanup. Fifty-gram subsamples of chopped soybean plants were homogenized with 80 milliliters of water and 100 milliliters of acetonitrile and filtered. Then 500 milliliters of 2 percent sodium chloride solution were added. The filtrate was extracted twice with 100 milliliters of petroleum ether, which was dried with anhydrous sodium sulfate, and concentrated to a volume suitable for analysis.

Insecticide residues were extracted from the soybean seeds by homogenizing 50 grams of the finely ground beans in 100 milliliters of 95 percent ethanol for 2 minutes and then for an additional 3 minutes after adding 100 milliliters of benzene. After filtration, the solid bean residue was extracted again with 100 milliliters of benzene. The organic solvent extract was washed three times with a 2 percent chloride solution; 500 milliliters for the first wash and 200 milliliters for the other washes. The washes were discarded, and the extract was dried with anhydrous sodium sulfate and concentrated to an oily residue. One-half of the oil, but not exceeding 5 milliliters, was removed and sufficient petroleum ether was added to bring the total volume to 25 milliliters. This was then extracted four times with 25-milliliter-portions of acetonitrile. To the combined acetonitrile extract was added 100 milliliters of petroleum ether and 500 milliliters of 2 percent sodium chloride solution. After thorough mixing, the aqueous layer was discarded, and the organic solvent fraction was washed twice with distilled water. The petroleum ether was dried with anhydrous sodium sulfate and concentrated to about 5 milliliters.

The concentrate was transferred to the top of a florisil column which had been prepared in the following manner. Florisil activated at 1093°C. and stored at 130°C. was added to a 2 x 30-centimeter column to a height of 4 inches. One-half inch of anhydrous sodium sulfate was placed on the florisil, and the column was washed with 50 milliliters of petroleum ether. After the extract was placed on the column, the insecticides were eluted with 300 milliliters of a 1:1 mixture of petroleum ether and ethyl ether. The eluate was then concentrated, and an aliquot was injected into the gas chromatograph.

RESULTS

Heptachlor-Treated Soils

Heptachlor applied to the soil at a rate of 3 pounds per acre resulted in average residues in the soil of 0.44 ppm 8 days after treatment (Table 1). Fifty percent of these residues were alpha-chlordane, 32 percent heptachlor and 18 percent heptachlor epoxide. Applying

TABLE 1. RESIDUES IN SOYBEANS GROWN IN SOILS TREATED WITH HEPTACHLOR PRIOR TO PLANTING

Insecticide residues	PPM residues in soils at planting, in soybean plants and in soybean oil ¹		
	Soil	Soybean plants ²	Soybean oil ³
3 pounds per acre			
Heptachlor	0.14(.11-.19)	<0.01	0.03(.02-.04)
Heptachlor epoxide	.18(.01-.17)	.02(.01-.04)	.38(.28-.49)
Alpha-chlordane	.22(.18-.28)	.01(.01-.02)	.07(.05-.10)
Total	.54	.03	.48
6 pounds per acre			
Heptachlor	0.34(.28-.41)	<0.01	0.06(.04-.13)
Heptachlor epoxide	.04(.02-.06)	.06(.04-.10)	.81(.38-1.3)
Alpha-chlordane	.55(.49-.63)	.01(.01-.02)	.08(.04-.15)
Total	.93	.07	.95

¹Values represent average ppm in eight samples, two samples from each of four field plots. The ranges are given in parenthesis.

²Whole plants harvested 140 days after planting.

³Oil constituted approximately 10 percent of soybean seeds. Mature seeds harvested 174 days after planting.

heptachlor at a rate of 6 pounds per acre gave residues of 0.93 ppm in the soil after 8 days. The composition of the residues were similar to that observed in soils treated with 3 pounds of heptachlor per acre. An assay of untreated soils (Table 3) showed that less than 0.1 ppm of the residues were present prior to the application of heptachlor for these studies.

Soybean plants grown in soils treated with 3 and 6 pounds of heptachlor per acre contained small amounts of insecticide residues when analyzed 140 days after planting (Table 1). Average total residues were 0.03 and 0.07 ppm for the two treatments, respectively. Heptachlor epoxide was the major component of the total residues while alpha-chlordane was present at about the 0.01 level. Only trace quantities of heptachlor were detected.

The insecticide content of beans from soybeans grown in heptachlor-treated soils is shown in Table 1. These data represent the ppm insecticide residues in the extracted bean oil which constituted approximately 10 percent of the weight of the whole beans. When calculated on this basis, it was found that the total residues in the oil, 0.48 and 0.95 ppm for treatment rates of 3 and 6 pounds per acre, were about the same level as detected in the soil 8 days after treatment. However, the relative concentrations of the three materials assayed were quite different. Whereas heptachlor epoxide was a minor residue in the soil, it was the major residue in the soybean oil. The combined residues of heptachlor and alpha-chlordane were only 20 percent of the total residues quantitated.

Obviously, heptachlor epoxide must be considered as a possible contaminant of soybean oil if the crop was grown in soils treated with heptachlor. This study indicates that the concentration of heptachlor epoxide

in the oil was approximately two and one-half times that level of heptachlor, per se, in the soil at planting. Additional investigations will be necessary to determine if this same factor would hold true for different soil types, different soybean varieties and different ambient conditions during the growing season.

Chlordane-Treated Soils

Total residues of alpha-chlordane, gamma-chlordane and heptachlor epoxide in the soil at the time soybeans were planted were 0.79 ppm following chlordane application at 8 pounds per acre (Table 2). When applied at 12 pounds per acre, the total residues were 0.84 ppm. Unfortunately, these two applications yield almost identical levels of residues in the soil rather than being significantly different as anticipated. The reason for this occurrence was not clear. However, it was apparent from agreement among replications that it was not caused by an uneven application of the spray. More than 90 percent of the quantitated residues in the soil were alpha- and gamma-chlordane. Only small quantities of heptachlor were detected while the concentration of heptachlor epoxide was always less than 0.01 ppm, the limit of sensitivity of the analytical method.

Analysis of whole soybean plants harvested at pod formation revealed total residues of 0.03 and 0.07 ppm when the plants were grown in soils treated with 8 and 12 pounds of chlordane per acre, respectively, (Table 2).

Oil from soybeans grown in soils treated with chlordane contained residues of alpha- and gamma-chlordane and heptachlor epoxide (Table 2). The concentration of gamma-chlordane was approximately one-half that of alpha-chlordane, and their sum was approximately one-fourth of the sum of their concentration in the soil

TABLE 2. RESIDUES IN SOYBEANS GROWN IN SOILS TREATED WITH CHLORDANE PRIOR TO PLANTING

Insecticide residues	PPM residues in soil at planting, in soybean plants and in soybean oil ¹		
	Soil	Soybean plants ²	Soybean oil ³
8 pounds per acre			
Alpha-chlordane	0.33(.22-.61)	0.02(.01-.03)	0.13(.08-.22)
Gamma-chlordane	.42(.28-.78)	.01(.01-.02)	.06(.05-.07)
Heptachlor	.04(.02-.07)	<.01	<.01
Heptachlor epoxide	<.01	<.01	.11(.07-.20)
Total	.79	.03	.30
12 pounds per acre			
Alpha-chlordane	0.35(.22-.49)	0.04(.03-.05)	0.18(.15-.22)
Gamma-chlordane	.42(.27-.60)	.03(.02-.03)	.07(.05-.12)
Heptachlor	.07(.03-.11)	<.01	<.01
Heptachlor epoxide	<.01	<.01	.17(.12-.20)
Total	.84	.07	.42

¹Values represent average ppm in eight samples, two samples from each of four field plots. The ranges are given in parenthesis.

²Whole plants harvested 140 days after planting.

³Oil constituted approximately 10 percent of soybean seeds. Mature seeds harvested 174 days after planting.

at planting. The level of heptachlor epoxide in the oil was about two and one-half times its concentration in the soil.

Chlordane-Treated Plants

Untreated soils in which soybeans were planted for studies involving foliar applications of chlordane contained low levels, 0.09 ppm total, of alpha- and gamma-chlordane (Table 3). These data represent the magnitude of residues in soils prior to the application of heptachlor and chlordane in the above studies. Heptachlor and heptachlor epoxide were below the level of sensitivity of the method. None of the pesticides considered in this study was detectable in soybean plants or seeds when grown in the untreated soil.

As expected, the level of residues in soybean plants was considerably greater following foliar application of chlordane than when the insecticide was applied to the soil. Plants sprayed with chlordane at a rate of 1 pound per acre had residues of 0.38 ppm when harvested 36

days after the last two treatments applied 7 days apart. Gamma-chlordane made up 57 percent of these residues, while the remainder was as alpha-chlordane. This same ratio was observed in plants treated with 3 pounds of chlordane per acre although the total residue content was 1.20 ppm. Heptachlor and heptachlor epoxide were not present in the plants at a detectable level.

Alpha-chlordane, gamma-chlordane and heptachlor epoxide were detected in the soybean oil. Oil from the crop sprayed with 1 pound of chlordane per acre contained a total of 0.62 ppm residues, and the oil from the crop treated at 3 pounds per acre contained 0.72 ppm. Approximately 45 percent of the residues were alpha-chlordane, 30 percent gamma-chlordane and 25 percent heptachlor epoxide. Apparently, the latter component was derived from heptachlor even though the parent compound was not evident in the soil at planting or in the soybean plants after foliar application of chlordane.

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TABLE 3. RESIDUES IN SOYBEANS SPRAYED TWICE WITH CHLORDANE¹

Insecticide residues	PPM residues in soil at planting, in soybean plants and in soybean oil ²		
	Soil ³	Soybean plants ⁴	Soybean oil ⁵
1 pound per acre			
Alpha-chlordane	0.02(.01-.03)	0.16(.09-.20)	0.25(.18-.31)
Gamma-chlordane	.07(.03-.10)	.22(.13-.27)	.21(.15-.30)
Heptachlor epoxide	<.01	<.01	.16(.14-.22)
Total	.09	.38	.62
3 pounds per acre			
Alpha-chlordane	0.04(.01-.06)	0.51(.28-.68)	0.34(.24-.39)
Gamma-chlordane	.05(.03-.07)	.69(.46-.92)	.23(.15-.26)
Heptachlor epoxide	<.01	<.01	.15(.11-.19)
Total	.09	1.20	.72

¹Second treatment applied 7 days after the first.

²Values represent average ppm in eight samples, two samples from each four field plots. The ranges are given in parenthesis.

³Soils sampled at time of planting, untreated.

⁴Whole plants harvested 36 days after the last insecticide application.

⁵Oil constituted approximately 10 percent of soybean seeds. Mature seeds harvested 70 days after last insecticide application.

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Residues in Corn and Soils Treated with Technical Chlordane and High-Purity Chlordane (HCS 3260)¹

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ABSTRACT

Mature corn grain and cobs grown in soils treated at planting with 1 and 2 lb active ingredient per acre of technical chlordane or HCS 3260 (high purity chlordane) were free of detectable insecticide residues (limit of sensitivity = 0.008 ppm). The stalk contained trace quantities of alpha- and/or gamma-chlordane. Whole corn plants harvested as for silage, 102 days after planting, con-

tained about 0.03–0.04 ppm combined alpha- and gamma-chlordane, while heptachlor and heptachlor epoxide were not detected. Maximum ppm total residues in the upper 4 inches of soil were approximately twice the treatment rate in pounds in the case of technical chlordane and 3–4 times the treatment rate of HCS 3260. Fifty to 70% of these residues had dissipated after 1 year.

The chemical composition of chlordane and its relationship to the toxicity of this insecticide has been a subject of considerable interest since the time it was introduced in the mid-1940's. Ingle (1965) presented the more pertinent points relative to this problem in his monograph on chlordane. As he pointed out, chlordane is not a definite chemical entity, and the various components comprising the technical material have not always appeared in the same proportion. The difficulty that this situation imposed on the analytical and toxicological evaluations of chlordane is obvious. Improved synthesis in 1951 yielded a more chemically consistent product and eliminated the presence of certain toxic intermediates and/or other impurities. Now another step in producing a chlordane of known chemical identity and free of toxic-related compounds has been undertaken by the manufacturers.

HCS 3260 is a high-purity chlordane which consists of 95+ % of alpha- and gamma-chlordane (Anonymous 1970). Gas chromatographic analysis of the product in our laboratory demonstrated that it was virtu-

ally void of other components sensitive to electron capture detection, whereas, technical chlordane contained these 2 isomers plus heptachlor and other related compounds (Fig. 1). Since HCS 3260 is essentially a new product as a result of improved synthesis, it is necessary that this high-purity chlordane be studied in detail insofar as its residual nature is concerned.

Depending upon the efficacy and toxicological nature of HCS 3260, there is a possibility that the compound would be of importance in controlling insect pests of corn. Since many of the more persistent insecticides once used for this purpose can no longer be employed, a definite need exists for a long-lasting, insecticidally active compound with low mammalian toxicity. HCS 3260 has certain characteristics which suggest that it may meet the needs of the corn producer in regard to efficacy and toxic properties. The residue situation which would accompany its use has not been demonstrated. Our current study evaluated the persistence of residues in the soil and in corn grown therein, when EC formulations of technical chlordane and HCS 3260 were applied at the time the crop was planted.

METHODS AND MATERIALS.—*Treatment.*—The plot area was plowed and prepared for planting in the spring of 1970. Plots 22×60 ft were selected for

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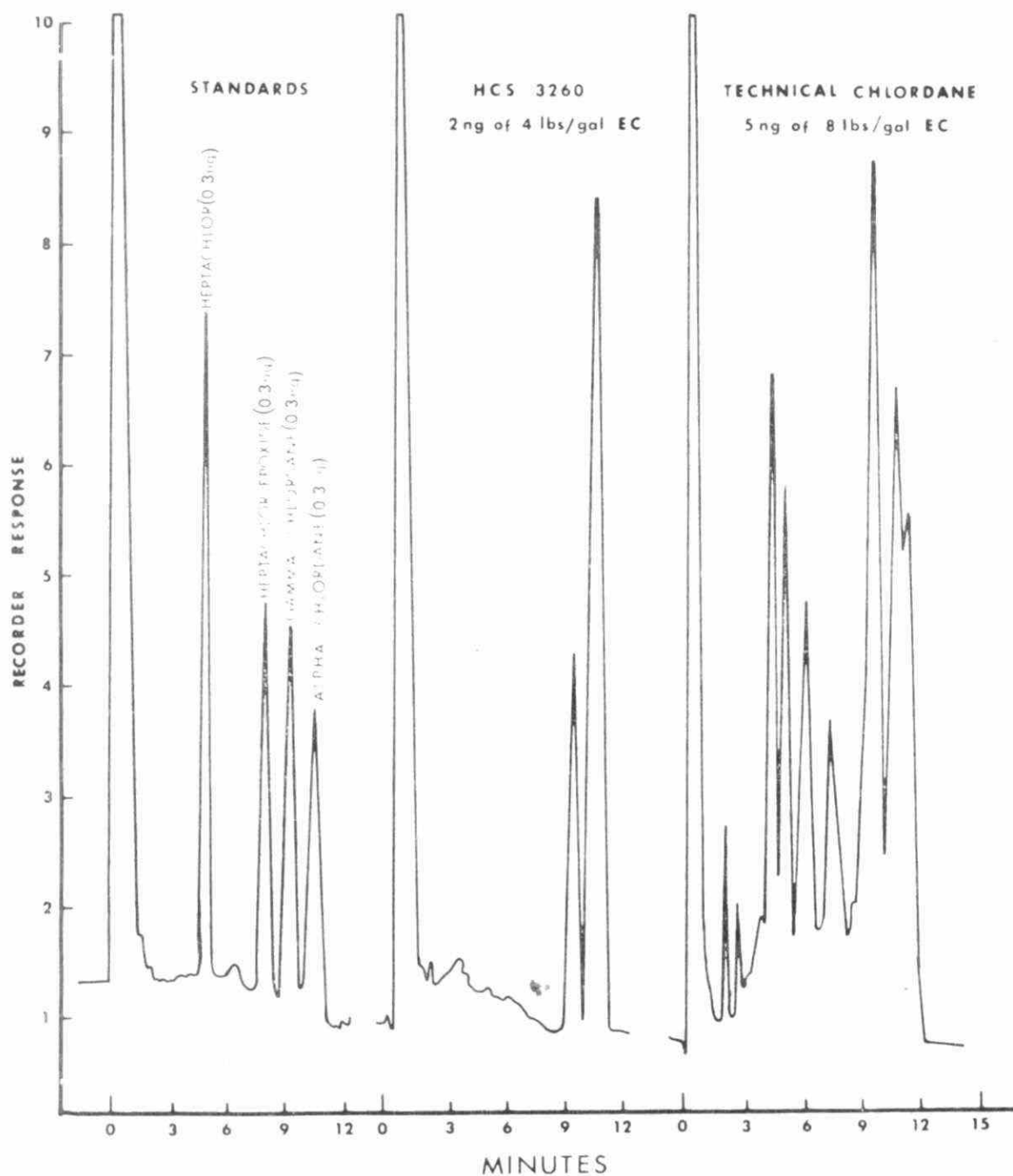


Fig. 1.—Gas chromatographic spectra of insecticide standards, HCS 3260, and technical chlordane on a DC-200 column.

treatment with either technical chlordane or HCS 3260 sprays. The EC formulations were diluted with sufficient water to obtain treatment rates of 1 or 2 lb AI/acre when applied in 20 gal of spray/acre. Applications were made with a low-pressure, low-volume, trailer-mounted John Bean Spartan® sprayer equipped with 13 Spraying Systems 730308 TeeJet® nozzles and operated at a pressure of 30 psi. The insecticides were applied May 8, 1970, and im-

mediately incorporated into the soil by disking to a depth of 2–4 in. A single-cross variety of corn (P.A.G. S×29) was planted the same day. Each treatment and the untreated check were replicated 3 times in a randomized complete block design.

Sample Collection.—Twenty soil cores, 1×4 in., were collected from each plot immediately before spraying and then 1 hr subsequent to the disking operation. Additional soil samples were collected 3,

6, and 12 months after treatment. The soil cores from each plot at each sampling date were mixed thoroughly, placed in sealed containers, and frozen until analyzed.

Samples consisting of 15 randomly selected stalks from each plot were cut 102 days following treatment. The "fresh cut" crop material was chopped as for silage, mixed thoroughly, and representative samples were taken from each plot and frozen immediately. The remainder of the corn was allowed to remain in the field until Oct. 19, 1970, before samples of the grain, cob, and stalk were collected and frozen.

Extraction and Cleanup.—All samples except the corn grain were extracted and prepared for GLC analysis using techniques described previously (Dorough et al. 1972). Briefly, this involved extracting the material with acetonitrile, transferring the pesticides to petroleum ether by partitioning in the presence of a sodium chloride solution, and then further cleanup of the extract on a Florisil column. The procedure for soils was similar but with the extracting solvent being a 1:1 mixture of benzene and acetone.

Corn grain was extracted and cleaned up just as described for the other plant material; however, analysis of treated grain showed several interfering peaks which required removal before quantitation of the chlordanes and heptachlors was possible. This was accomplished by subjecting the eluate from the Florisil column to TLC analysis. A 25-g equivalent of corn grain was applied to a Chromar 500 TLC sheet (Malkinkrodt, St. Louis, Mo.) and developed in hexane. Areas of the sheet corresponding to the position of the pesticide standards were cut from the sheet and extracted thoroughly with acetone. The solvent was removed by evaporation, the residue was taken up in hexane, and aliquots of the hexane solution containing 0.025-g equivalents of grain were injected into the gas chromatograph.

TLC of the corn grain extract successfully separated materials which interfered with the quantitation of heptachlor, heptachlor epoxide, and gamma-chlordane. There remained, however, an interfering material with an apparent identical retention time

as alpha-chlordane. Only when an extract of a control sample of grain was fortified with alpha-chlordane was it evident that alpha-chlordane peaked about 1 mm before the unknown material from untreated corn. Repeated fortification of the control sample with various concentrations of alpha-chlordane revealed that a level of sensitivity of 0.008 ppm could be obtained even in the presence of the interfering product.

Gas Chromatographic Analysis.—A Varian Aerograph Series 1700 gas chromatograph equipped with an electron capture detector was used to quantitate the insecticide residues. The glass column, 6 ft x 1/8 in. ID, was packed with 10% DC 200 on 80-90 mesh Anakron ABS. Nitrogen at 45 ml/min served as the carrier gas, and the temperatures were: column 195°C; injection port 200°C; detector 215°C. The retention times, in minutes, for heptachlor, heptachlor epoxide, gamma-chlordane, and alpha-chlordane were 5.4, 8.7, 10.2, and 11.6, respectively.

RESULTS AND DISCUSSION.—Recovery.—Recoveries of alpha-chlordane, gamma-chlordane, heptachlor, and heptachlor epoxide, when added at the 0.1 ppm level to soil were 90, 99, 89, and 93% respectively; for silage 73, 69, 93, and 68%; for grain 92, 88, 90, and 101%; for cob 96, 95, 89, and 95%; and for stalk 90, 98, 91, and 98%. The data were not corrected for loss of pesticide during extraction and cleanup.

The 4 insecticidal materials listed in Table 1 were the only components for which quantitation was attempted. Since 60-70% of technical chlordane consisted of compounds other than those quantitated (Fig. 1), the residue values given in this study represent only about 1/3 the total technical chlordane equivalents which could be present (Dorough et al. 1972). With the HCS-3260-treated materials, the reported values reflect total residues, because only 2 products were present in the applied spray, and both are known materials which can be quantitated.

Soils.—Heptachlor epoxide was not detected in soils sampled either immediately after treatment or at any time thereafter (Table 1). There were, however, small amounts of heptachlor in soils receiving technical chlordane treatment of 1 and 2 lb/acre. The concentration of heptachlor never exceeded 0.012

Table 1.—Residues in soils of corn plots treated with EC formulations of chlordane and HCS 3260.

Insecticides detected	Ppm (dry wt) at indicated months after treatment with chlordane (C) or with HCS 3260 (H)							
	0		3		6		12	
	C	H	C	H	C	H	C	H
<i>1 lb/acre</i>								
α-Chlordane	0.080	0.196	0.056	0.058	0.074	0.273	0.046	0.195
γ-Chlordane	.066	.074	.054	.114	.077	.097	.037	.086
Heptachlor	.006	0	.009	0	0	0	0	0
Total	.152	.270	.119	.372	.151	.370	.083	.281
sd*	.036	.088	.048	.106	.051	.080	.040	.027
<i>2 lb/acre</i>								
α-Chlordane	.079	.426	.098	.673	.087	.537	.069	.240
γ-Chlordane	.082	.148	.084	.247	.090	.224	.062	.092
Heptachlor	.012	0	.010	0	0	0	0	0
Total	.173	.574	.192	.820	.177	.761	.131	.332
sd	.056	.294	.070	.096	.092	.017	.015	.033

* sd for total residue.

Table 2.—Residues in corn plants and grain grown in soils treated with EC formulations of chlordane and HCS 3260.^a

Plant material and treatment rates	Ppm residues ^b (dry wt)							
	Chlordane				HCS 3260			
	Alpha chlordane	Gamma chlordane	Total	SD ^c	Alpha chlordane	Gamma chlordane	Total	SD ^c
<i>Silage</i> ^d								
1 lb/acre	0.019	0.013	0.032	0.010	0.017	0.012	0.029	0.020
2 lb/acre	.021	.013	.034	.006	.028	.015	.043	.002
<i>Grain</i> ^e								
1 lb/acre	0	0	0	0	0	0	0	0
2 lb/acre	0	0	0	0	0	0	0	0
<i>Cob</i> ^e								
1 lb/acre	0	0	0	0	0	0	0	0
2 lb/acre	0	0	0	0	0	0	0	0
<i>Stalk</i> ^e								
1 lb/acre	.008	0	.008	.005	0	0	0	0
2 lb/acre	.011	.009	.020	.004	.008	0	.008	.006

^a Soil treated May 8, 1970, with either 1 or 2 lb/acre insecticide. After disking into soil, the corn was planted.^b Level of sensitivity = 0.008 ppm.^c SD for total residue.^d Harvested 102 days after planting, chopped as for silage, and frozen immediately.^e Harvested 164 days after planting.

ppm and was not detected in samples taken 3 months post-treatment.

The major residues in soils following applications of both technical chlordane and HCS 3260 were alpha- and gamma-chlordane. With the technical material the 2 isomers were present in 0-time soils in about equal quantities, 0.07–0.08 ppm. In soil treated with HCS 3260 the alpha-chlordane existed in concentrations about 3-fold that of the gamma isomer. These ratios of insecticidal components in the soil immediately after application were in accordance with their content in the EC formulations (Fig. 1).

Estimation of the dissipation rates of the insecticides was complicated by the detection of higher levels of residues in the 3-month sample than in the 0-time soils. However, if the 3-month values are taken as the maximum residues present, then at the 12-month sampling interval, the total loss of insecticidal materials was generally between 50 and 70%.

Because of the inconsistencies in the residue data on soils, the treatments were repeated on a smaller scale. Treatment rates and methods were identical to the 1st tests, but soil samples were collected at monthly intervals for 3 months. Results of these experiments showed that soils treated with technical chlordane at 1 and 2 lb/acre contained 0.08 and 0.17 ppm residues at 0 time. By 1 month the level of residues in the soil had declined by 35% and by 2 months between 70 and 75% of the materials had dissipated. Only 5% decline of the residues was noted between the 2nd and 3rd months after treatment.

Samples taken after disking the HCS 3260 insecticide into the upper 2–4 in. of soil showed a total deposit of 0.71 and 1.02 ppm from the 2 treatment rates. These concentrations of alpha- and gamma-

chlordane declined by 75% after only 1 month and then persisted at approximately the same levels for the next 2 months.

Basically, the 2nd test on the persistence of chlordane and HCS 3260 in soils served only to further demonstrate the variability inherent in studying the residual nature of pesticides in soils. However, these data did agree with those of the 1st test, which suggested that as much as 70% of the applied materials would dissipate within 1 year after application.

Corn.—Whole corn plants harvested at the proper growing stage for making silage contained residues of alpha- and gamma-chlordane. Heptachlor and heptachlor epoxide were not present at detectable levels (Table 2). These data indicated that corn produced for silage would contain 0.0% and 0.04 ppm chlordane residues at the time of harvest if the soil had been treated with 1 or 2 lb/acre technical chlordane or HCS 3260. Corn grain and cob were free of detectable levels of insecticides, and the stalk contained alpha- and/or gamma-chlordane at levels near the limit of sensitivity of the method, 0.008 ppm (Table 2).

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Residues in Alfalfa and Soils Following Treatment with Technical Chlordane and High Purity Chlordane (HCS 3260) for Alfalfa Weevil Control

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Alfalfa was sprayed with technical chlordane and with a high purity chlordane (HCS 3260) which contained 98+ % α - and γ -chlordane. The active ingredients (AI) of technical chlordane consisted of 14% each of α - and γ -chlordane, 5% heptachlor, and 67% related compounds. Analyses were conducted for α -chlordane, γ -chlordane, heptachlor, and heptachlor epoxide which considered over 98% of the AI of HCS 3260 but only 33% of the AI of technical chlordane. Total ppm of these products

deposited on alfalfa treated with the technical chlordane and HCS 3260 at a rate of 1 lb AI/acre were 29 and 130, respectively. In all tests, 95% of the residues on the alfalfa had disappeared after 21 days. Three days of field-curing freshly cut alfalfa reduced the level of residues by about 55%. Maximum total residues were 0.6 ppm in soil of alfalfa treated with 2 lb AI/acre of HCS 3260. By 21 days these residues had declined by 50%, and by 180 days 83% of the residues had dissipated.

Technical chlordane is a complex mixture of polychlorinated bicyclohydrocarbons consisting of 60 to 75% isomers of chlordane and 25 to 40% of related compounds (Martin, 1968). Modern chromatographic techniques allow the separation of technical chlordane into a series of components which are extremely sensitive to electron capture detection. Using glc parameters described later in this paper, at least nine such products were resolved (Figure 1) from technical chlordane produced by Velsicol Chemical Corp. under the name Belt-72ECF.

Problems created by the presence of a variety of components in a biologically active material in regard to evaluation of its chemical and toxicological properties are apparent. In fact, this has been a subject of interest and periodic concern with chlordane since its introduction in 1945 (Ingle, 1965). A related problem, and one which has particular significance because it hinders our ability to monitor the environment and food products for pesticides residues, is the difficulty encountered in attempting to quantitate total chlordane residues by glc techniques.

While it has been possible for several years to obtain excellent recovery of chlordane from fortified samples, several workers have shown that weathered residues change in the number and size of gas chromatographic peaks (Klein and Link, 1967; Thurston, 1965). Estimates of the total level of residues on a weathered substrate were based on an average concentration calculated from several of the glc peaks. More recently, certain known components of chlordane (α -chlordane, γ -chlordane, heptachlor and its metabolite, heptachlor epoxide) were selected for quantitation in soils and plant material (Dorough *et al.*, 1969). However, no totally satisfactory method for quantitating technical chlordane residues has been devised.

Possibly, the need for developing such a method has been lessened. Velsicol Chemical Corp. recently developed a high purity chlordane reported to contain 95% or more of the α and γ isomers of chlordane (Velsicol Chem. Corp., 1970). This product is identified by Velsicol as HCS 3260, and is

used to identify the product in the text and tables of this paper.

Glc analysis of HCS 3260 in our laboratory showed that the product was comprised almost entirely of γ -chlordane, retention time 10.2 min, and α -chlordane, retention time 11.6 min (Figure 1). Since analytical reference standards for these two isomers are available, the problem of quantification one encountered with technical chlordane no longer applies. The effect of the purification of technical chlordane on its insecticidal effectiveness and residual persistence was evaluated in the current study.

METHODS AND MATERIALS

Treatment. Field plots (20 \times 60 ft) of alfalfa (Narragansett variety), were selected for treatment with either technical chlordane or HCS 3260 sprays. The EC formulations, containing 8 and 4 lb of active ingredient (AI) per gal, respectively, were diluted with sufficient water to obtain treatment rates of 1 or 2 lb/acre (AI) when the crop was treated with 20 gal of spray per acre. Treatments were applied with a portable boom-type, compressed-air, hand sprayer equipped with four Spraying Systems 730308 TeeJet nozzles and operated at 30 psi. In the first experiment, applications were made on May 4, 1970, to alfalfa approximately 12 in. high. Identical applications were made to other alfalfa plots on September 11, 1970, when this crop was about 6 in. tall. Each treatment and the untreated check were replicated three times in a randomized complete block design. During the 21 days after the May treatment, the total rainfall was 1.8 in. and the average temperature was 19° C. For the same interval after the September treatment, there were 3.8 in. of rain and the average temperature was 23° C. The soil type in the treated area was Armor silt loam.

Sample Collection. Twenty soil cores, 1 \times 4 in., were collected at random from each plot immediately before spraying the alfalfa and then 1 hr subsequent to the spraying operation. Additional soil samples were collected 21, 90, and 180 days after treatment. The soil cores from each plot were mixed thoroughly, placed in sealed containers, and frozen until analyzed.

The alfalfa was cut 21 days following treatment and representative samples of the "fresh cut" crop material were taken

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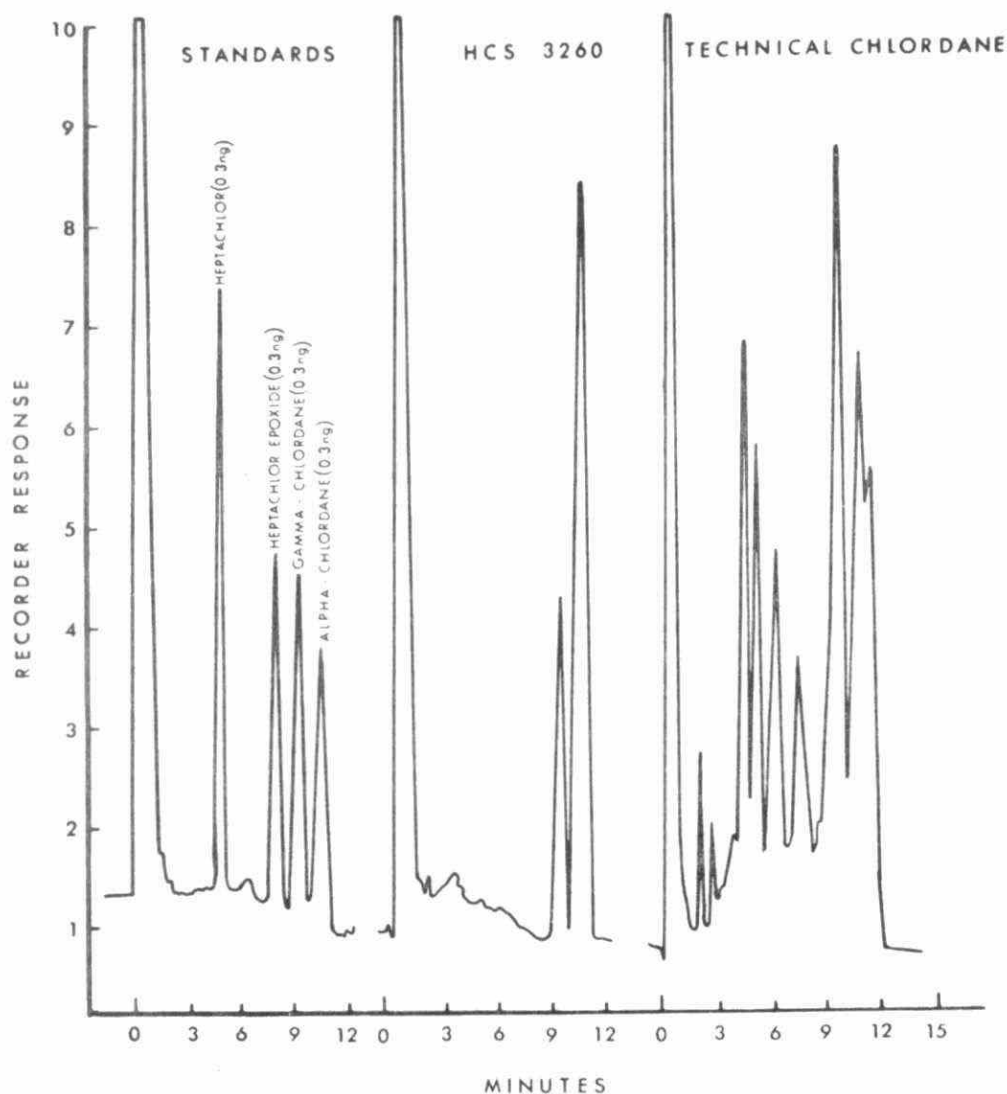


Figure 1. Gas chromatographic spectra of insecticide standards, HCS 3260 (2 ng of 4 lb/gal EC) and technical chlordane (5 ng of 8 lb/gal EC) on a DC-200 column. See methods section for complete operating parameters

from each plot and frozen immediately. The remainder of the cut alfalfa was allowed to remain in the field for 3 days before samples of the plants were collected and frozen. The latter samples are referred to as "field cured" alfalfa in the text and tables.

Prior to analysis, subsamples of the soils and alfalfa from each replication were oven-dried to a constant weight and the water content was calculated. No adjustment in the water content of field-collected samples was made for analysis; however, all data are expressed on the basis of the dry weight of the samples.

Extraction and Clean-up. All solvents used in these investigations were MC/B pesticide-quality materials. Insecticides were extracted from the alfalfa plants with acetonitrile. A 50-g sample was blended twice with the solvent, the volume being 300 ml and 150 ml, respectively, for the two extractions. Exactly one-half of the combined acetonitrile extracts, after filtering, was transferred to a 1-l. separatory funnel which contained 100 ml of petroleum ether and the mixture was shaken.

Five-hundred milliliters of a 2% sodium chloride solution were introduced into the funnel, the funnel was shaken thoroughly, and the phases were allowed to separate. The aqueous layer was again extracted with 100 ml of petroleum

ether and the ether extracts were combined and dried with anhydrous sodium sulfate. Next, the extract was filtered and concentrated to a volume of 2 ml for column cleanup.

A glass chromatographic column (20 mm i.d.), filled with 20 g of florisil, 60/100 mesh, and a 1-in. layer of anhydrous sodium sulfate, was prewashed with 10% diethyl ether in petroleum ether. The 2 ml extract of the alfalfa plants was added to the florisil column and the insecticides were eluted with 200 ml of the 10% diethyl ether solution. Normally, the eluate was concentrated to 10 ml for gas chromatographic analysis. When necessary to attain the desired sensitivity, the samples were further concentrated.

For extraction of the insecticides from soils, a 100-g sample was added to a quart jar containing 200 ml of a 1:1 mixture of benzene and acetone. The sample was then placed on an Eberbach flatbed shaker and shaken at 180 cpm for 1 hr. The solvent was decanted completely from the soil and passed through Whatman No. 1 filter paper into a 500-ml separatory funnel. A 2% sodium chloride solution, 200 ml, was then added and the contents were thoroughly shaken. After allowing the two phases to separate, the aqueous layer was extracted with 100 ml of benzene, which were then combined with the benzene from the initial soil extraction. The benzene was dried with anhydrous sodium sulfate, concentrated to a

volume of 2 ml, and subjected to a florisil column cleanup step as described for plant material. Aliquots of the concentrated eluate were injected into the gas chromatograph.

Gas Chromatographic Analysis. Each extract of soil or alfalfa was subjected to gas chromatographic analysis using Varian Aerograph Series 1700 instruments equipped with electron capture detectors. The following conditions were used.

	Column A	Column B
Column	Glass, 6 ft \times $\frac{1}{8}$ in. i.d.	5 ft \times $\frac{1}{8}$ in. i.d.
Packing	DC 200, 10% (w/w) on 80 to 90 mesh Anakrom ABS	SE 30, 3% (w/w) on 90 to 100 mesh Chromosorb P
Carrier gas	Nitrogen, 45 ml per min	Nitrogen, 25 ml per min
Temperatures	Column 195° C, injection port 200° C, detector 215° C	Column 200° C, injection port 200° C, detector 200° C

Recovery Tests. Analytical standards of α -chlordane, γ -chlordane, heptachlor, and heptachlor epoxide (Velsicol Chemical Corp., Chicago, Ill.) were used to determine the efficiency of the extraction procedures described above. Each material was added to the substrate, soil, or alfalfa at the 0.1-ppm level prior to the addition of the first extracting solvent. Recovery experiments were run on the individual insecticides and with a mixture of the four products. In addition to these recovery tests, the completion of extraction of aged residues from the soil and alfalfa was determined by re-extracting the solids after the normal procedure with the same and with different solvents, and analyzing these extracts separate from the original. Only when additional extractions failed to yield significant residues were the original procedures accepted.

Volatilization Experiments. The dissipation of α - and γ -chlordane from field-treated alfalfa was investigated under laboratory conditions. A series of samples of fresh cut alfalfa, collected 21 days after receiving a 1 lb/acre treatment of HCS 3260 on May 4, 1970, were spread evenly over a flat surface and allowed to remain at room temperature for a total of 14 days. At predetermined intervals, subsamples of the plant material were analyzed for pesticide and for moisture content.

To ascertain the nature of the escaped residues, similar samples of alfalfa were placed in containers designed so that air was passed over the plant material and into a cold hexane trap. The trap was operated for 8 hr and the nature and quantity of residues in the hexane were determined using the glc procedures already described.

Biological Activity. Surveys of the treated and untreated plots for alfalfa weevil larvae were made by taking 10 net sweeps/plot on 3, 7, 14, and 21 days after spraying the alfalfa on May 4, 1970. Also, an estimate of the insect damage was made by visual inspection of the crop and assigning a damage rating to each treatment. On the 21st day after treatment, the alfalfa was harvested and yield data were collected from the treated and untreated plots.

RESULTS AND DISCUSSION

Components of EC Formulations. Figure 1 shows that the technical chlordane was composed of at least nine components sensitive to electron capture detection. Although no attempt

was made to characterize each component, it was established that α -chlordane and γ -chlordane were present and that each accounted for approximately 14% of the total active ingredients. Heptachlor accounted for about 5% of the total active ingredients. The purified chlordane, HCS 3260, contained at least 74% α -chlordane and 24% γ -chlordane. By increasing the quantity of HCS 3260 injected into the glc by 100-fold that amount necessary for full scale deflection for the γ -chlordane peak, trace quantities of a material with the same retention time as heptachlor were noted. Its identity was not confirmed.

Considering the α and γ isomers as the only active ingredients in the EC formulations and calculating the lb/acre rates on a total AI basis revealed that applications of the HCS 3260 material resulted in five times more α -chlordane and two times more γ -chlordane than when the technical chlordane was applied. Thus, when 1 lb of AI/acre was desired, the actual α - + γ -chlordane applied was only 0.3 lb/acre in the case of technical chlordane but approximately the calculated rate of lb/acre for the HCS 3260. This is an important consideration in analyzing the data presented herein since residues reported in the soil and alfalfa were based only on the content of α -chlordane, γ -chlordane, heptachlor, and heptachlor epoxide. Total active ingredients on the technical chlordane-treated substrates were not quantitated.

One should keep in mind, then, that "total residues" means total active ingredients when referring to the HCS 3260 treated materials because the α and γ isomers were the only observed components of the AI. However, "total residues" in the technical chlordane treated soils and alfalfa refer only to the above stated compounds and their sum would have to be multiplied by a factor of approximately 3 to gain an estimate of the total "technical chlordane" present. This, of course, would require the assumption that all components of the technical chlordane (Figure 1) dissipated at the same rate. Detailed comparison of the 0-day and 21-day samples of soil and alfalfa showed that the major peaks generally maintained their relative peak heights even after considerable dissipation of the initial deposits had occurred. Therefore, this type of estimation of total technical chlordane residues very closely approximates the actual levels present in the substrates.

Recovery. The glc retention times of the four materials considered in the quantitation of residues in soil and alfalfa are shown in Table I. All of these materials were detectable

Table I. Glc Retention Time and Average Percentage Recoveries from Alfalfa and Soils of Chlordane and Related Compounds

Glc column ^a and substrate	Retention time, min, and average percent (\pm SE) recovery ^b			
	Heptachlor	Heptachlor epoxide	γ -chlordane	α -chlordane
DC 200 (10%)	5.4	8.7	10.2	11.6
SE 30 (3%)	7.1	11.7	12.7	14.0
Recovery from alfalfa	87 \pm 7	86 \pm 8	91 \pm 18	98 \pm 12
Recovery from soils	94 \pm 10	89 \pm 11	85 \pm 10	89 \pm 8

^a Glc column and operating parameters given in methods. ^b Samples fortified at the 0.1-ppm level prior to extraction.

Table II. Residues in Soils after Field Treatments of Alfalfa in May with EC Formulations of Chlordane and HCS 3260^a

Treatment and elapsed time ^c	Average ppm (\pm SE) at indicated rate (dry wt) ^b					
	α -Chlordane		γ -Chlordane		Total	
	1 lb/acre	2 lb/acre	1 lb/acre	2 lb/acre	1 lb/acre	2 lb/acre
1 hr (25.7)						
Chlordane	0.004 \pm 0.002	0.013 \pm 0.006	0.002 \pm 0.001	0.011 \pm 0.006	0.006 \pm 0.003	0.024 \pm 0.011
HCS 3260	0.024 \pm 0.006	0.027 \pm 0.012	0.009 \pm 0.003	0.010 \pm 0.008	0.033 \pm 0.009	0.037 \pm 0.016
21 days (24.5)						
Chlordane	0.005 \pm 0.002	0.008 \pm 0.001	0.004 \pm 0.002	0.007 \pm 0.001	0.009 \pm 0.003	0.015 \pm 0.003
HCS 3260	0.021 \pm 0.003	0.055 \pm 0.003	0.007 \pm 0.001	0.019 \pm 0.009	0.028 \pm 0.004	0.074 \pm 0.035
90 days (22.2)						
Chlordane	0.007 \pm 0.004	0.010 \pm 0.003	0.005 \pm 0.003	0.008 \pm 0.002	0.012 \pm 0.006	0.018 \pm 0.005
HCS 3260	0.022 \pm 0.020	0.034 \pm 0.010	0.006 \pm 0.002	0.016 \pm 0.004	0.028 \pm 0.009	0.050 \pm 0.013
180 days (27.0)						
Chlordane	0.066 \pm 0.004	0.010 \pm 0.007	0.003 \pm 0.001	0.009 \pm 0.006	0.009 \pm 0.006	0.019 \pm 0.006
HCS 3260	0.022 \pm 0.010	0.042 \pm 0.034	0.006 \pm 0.003	0.012 \pm 0.009	0.028 \pm 0.013	0.054 \pm 0.044

^a Alfalfa, 12 in. high, treated with technical chlordane or HCS 3260, a high purity chlordane containing 95% or more of α - and γ -chlordane, in a total spray volume of 20 gal/acre. Treated May 4, 1970. ^b No heptachlor or heptachlor epoxide (<0.001 ppm) detected in these soils. Data are averages from three field replications. ^c Numbers in parentheses indicate the average percent of water in samples.

Table III. Residues in Soils after Field Treatments of Alfalfa in September with EC Formulations of Technical Chlordane and HCS 3260^a

Treatment and elapsed time ^c	Average ppm (\pm SE) at indicated rate (dry wt) ^b					
	α -Chlordane		γ -Chlordane		Total	
	1 lb/acre	2 lb/acre	1 lb/acre	2 lb/acre	1 lb/acre	2 lb/acre
1 hr (15.4)						
Chlordane	0.053 \pm 0.001	0.105 \pm 0.015	0.049 \pm 0.002	0.100 \pm 0.013	0.102 \pm 0.003	0.205 \pm 0.029
HCS 3260	0.238 \pm 0.043	0.383 \pm 0.147	0.084 \pm 0.020	0.172 \pm 0.038	0.322 \pm 0.062	0.555 \pm 0.181
21 days (21.7)						
Chlordane	0.024 \pm 0.003	0.040 \pm 0.014	0.024 \pm 0.003	0.041 \pm 0.016	0.048 \pm 0.008	0.081 \pm 0.030
HCS 3260	0.135 \pm 0.010	0.199 \pm 0.048	0.055 \pm 0.005	0.077 \pm 0.020	0.190 \pm 0.011	0.276 \pm 0.068
90 days (23.8)						
Chlordane	0.015 \pm 0.003	0.024 \pm 0.004	0.013 \pm 0.002	0.021 \pm 0.004	0.028 \pm 0.005	0.045 \pm 0.007
HCS 3260	0.055 \pm 0.005	0.103 \pm 0.030	0.024 \pm 0.004	0.035 \pm 0.013	0.079 \pm 0.009	0.138 \pm 0.043
180 days (25.8)						
Chlordane	0.004 \pm 0.001	0.009 \pm 0.003	0.005 \pm 0.001	0.010 \pm 0.004	0.009 \pm 0.001	0.019 \pm 0.007
HCS 3260	0.044 \pm 0.020	0.064 \pm 0.017	0.018 \pm 0.007	0.028 \pm 0.006	0.062 \pm 0.027	0.092 \pm 0.023

^a Soils from alfalfa, 6 in. high, plots sprayed with technical chlordane or HCS 3260 in a total volume of 20 gal/acre. HCS 3260 is a high purity chlordane containing 95+ % α - and γ -chlordane. Treated September 11, 1970. ^b No heptachlor or heptachlor epoxide (<0.001 ppm) detected in these soils. Data are averages from three field replications. ^c Numbers in parentheses indicated average percent of water in soils.

at very low levels using the electron capture detector (Figure 1). With control samples, the levels of interfering materials in the soils and alfalfa were sufficiently low so that sensitivity levels of 0.001 and 0.004 ppm, respectively, were easily attainable.

Average recoveries of α - and γ -chlordane heptachlor, and heptachlor epoxide from soil and alfalfa were 85% or better. Variations in the percentage recovery of the different compounds were considerable (Table I), but do represent the situation as it occurred over a 4-month period of analysis since each set of samples analyzed daily included at least one recovery experiment.

Soils. Insecticide residues reaching the soil from the treatment of alfalfa in May consisted only of α - and γ -chlordane and were about $1/10$ those in the soil when the crop was sprayed in September (Tables II and III). This probably resulted because of the increased ground cover provided by the alfalfa in the first treatment as compared to that in the second. As noted earlier, the plants sprayed in May were approximately 1 ft in height while those sprayed in September

were about one-half that height. Maximum residues, 0.6 ppm, were deposited on the soils sprayed in September with 2 lb/acre of HCS 3260 (Table III). When calculated on a 4-in. acre basis, this accounted for 40% of the applied AI.

Dissipation of the α - and γ -chlordane occurred very slowly, if at all, during the 180-day period following the treatments in May. Factors contributing to such slow disappearance of the pesticides were the rapid regrowth of plants following harvest 21 days after treatment, very little rainfall, and a rather low average daily temperature. In the second test, the level of residues dropped about 50% during the first 21 days after treatment in September. The level of residues continued to decline and approximated those from the May treatment when analysis of each was conducted on samples collected 180 days after treatment. The more rapid dissipation of residues from the soil after the second treatment was likely facilitated by the higher temperatures and greater rainfall than occurred in the experiment initiated in May.

Alfalfa. There was excellent agreement of the data relative to the level of residues on alfalfa 21 days following treatment

Table IV. Residues in Alfalfa 21 Days after Treatments in May with EC Formulations of Technical Chlordane and HCS 3260 at Rates of 1 and 2 lb/acre^a

Treatment and sample ^c	Average ppm (±SE) in fresh cut and field-cured alfalfa (dry wt) ^b				Total
	α-Chlordane	γ-Chlordane	Heptachlor	Heptachlor epoxide	
			1 lb per acre		
Fresh cut (77.8)					
Chlordane	1.32 ± 0.56	1.08 ± 0.64	<0.001	0.25 ± 0.09	2.65 ± 1.30
HCS 3260	4.11 ± 1.31	1.35 ± 0.31	<0.001	<0.001	5.46 ± 1.33
Field-cured (30.5)					
Chlordane	0.39 ± 0.03	0.31 ± 0.08	<0.001	0.09 ± 0.02	0.79 ± 0.09
HCS 3260	2.24 ± 0.84	0.71 ± 0.25	<0.001	<0.001	2.95 ± 1.08
			2 lb per acre		
Fresh cut (77.8)					
Chlordane	2.03 ± 1.02	1.99 ± 1.11	<0.001	0.52 ± 0.35	4.54 ± 2.55
HCS 3260	11.72 ± 4.10	3.72 ± 2.78	<0.001	<0.001	15.44 ± 11.37
Field-cured (30.5)					
Chlordane	0.60 ± 0.11	0.64 ± 0.15	<0.001	0.16 ± 0.08	1.40 ± 0.32
HCS 3260	2.54 ± 1.58	0.68 ± 0.55	<0.001	<0.001	3.22 ± 2.33

^a Alfalfa, 12 in. high, treated with 20 gal/acre technical chlordane spray or with HCS 3260 spray, a high purity chlordane containing 95+% α - and γ -chlordane. Treated May 4, 1970. ^b "Fresh cut" samples were frozen immediately after cutting. "Field-cured" samples remained in the field for 3 days before freezing. Data are averages from three field replications, two analyses per replication. ^c Numbers in parentheses indicate average percent of water in samples.

Table V. Residues on Alfalfa 1 Hour and 21 Days Following Treatments in September with EC Formulations of Chlordane and HCS 3260 at Rates of 1 and 2 lb/acre^a

Treatment and elapsed time ^c	Average ppm (±SE) in fresh cut and field-cured alfalfa (dry wt) ^b					Total
	α-Chlordane	γ-Chlordane	Heptachlor 1 lb per acre	Heptachlor epoxide		
1 hr, fresh cut (76.3)						
Chlordane	13.64 ± 1.63	13.91 ± 2.04	1.77 ± 0.06	<0.001		29.32 ± 3.73
HCS 3260	96.56 ± 6.36	33.39 ± 6.83	0.17 ± 0.04	<0.001		130.12 ± 12.90
21 days, fresh cut (76.0)						
Chlordane	0.76 ± 0.08	0.71 ± 0.07	<0.001	0.23 ± 0.04		1.70 ± 0.17
HCS 3260	5.60 ± 0.40	1.75 ± 0.37	<0.001	<0.001		7.35 ± 1.12
21 days, cured (16.3)						
Chlordane	0.39 ± 0.04	0.40 ± 0.01	<0.001	0.15 ± 0.02		0.94 ± 0.08
HCS 3260	2.16 ± 0.78	0.61 ± 0.07	<0.001	<0.001		2.77 ± 0.80
2 lb per acre						
1 hr, fresh cut (76.3)						
Chlordane	34.23 ± 4.74	35.35 ± 4.84	6.88 ± 1.18	<0.001		76.46 ± 10.45
HCS 3260	453.72 ± 68.91	163.28 ± 15.53	0.65 ± 0.11	<0.001		617.65 ± 72.39
21 days, fresh cut (76.0)						
Chlordane	1.26 ± 0.27	1.34 ± 0.54	0.05 ± 0.01	0.38 ± 0.09		3.03 ± 0.87
HCS 3260	18.60 ± 1.84	5.79 ± 0.75	<0.001	<0.001		24.39 ± 2.69
21 days, cured (16.3)						
Chlordane	0.61 ± 0.24	0.57 ± 0.22	0.17 ± 0.07	<0.001		1.35 ± 0.51
HCS 3260	8.15 ± 2.54	2.51 ± 1.23	<0.001	<0.001		10.66 ± 4.18

^a Alfalfa, 6 in. high, treated with 20 gal/acre technical chlordane spray or HCS 3260 spray, a high purity chlordane containing 95+% α - and γ -chlordane. Treated September 11, 1970. ^b "Fresh cut" samples were collected immediately after cutting and frozen until analyzed. "Field-cured" samples were allowed to remain in the field for 3 days after cutting and then frozen until analyzed. Data are averages from three field replications, two analyses per replication. ^c Numbers in parentheses represent the average percent of water in samples.

Table VI. Comparative Concentrations of α - and γ -Chlordane in EC Formulations of Technical Chlordane and HCS 3260 and in Alfalfa Sprayed with These Insecticides

Sample ^b	Ratios ^a				
	T α /T γ	H α /H γ	H α /T α	H γ /T γ	H α + γ / T α + γ
EC formulations	1.0	2.6	6.0	2.2	4.0
Alfalfa, 1 hr, fresh cut, 1 lb/acre	1.0	2.9	7.1	2.3	4.7
Alfalfa, 21 day, fresh cut, 1 lb/acre	1.1	3.2	7.4	2.5	5.0
Alfalfa, 21 day, field-cured, 1 lb/acre	1.0	3.5	5.5	1.5	3.5

^a T = technical chlordane, 8 lb AI per gal. H = HCS 3260, 4 lb AI per gal. α = alpha-chlordane; γ = gamma-chlordane. ^b Data pertaining to alfalfa taken from Table V.

Table VII. Loss of Residues at Room Temperature from Freshly Cut Alfalfa Sprayed 21 Days Prior to Cutting with 2 lb/acre of HCS 3260^a

Residue	Initial ppm	Percent loss of residues after indicated days ^b		
		3	7	14
α -Chlordane	4.22	57.4	63.2	62.0
γ -Chlordane	1.10	51.6	59.1	68.4
Total	5.32	52.8	61.0	67.2

^a HCS 3260, a high purity chlordane containing 95+% α - and γ -chlordane. ^b Ppm residues at each time interval were calculated on the basis of the original weight of the sample (50 g) although the weight of the sample was reduced 70% in 3 days as a result of water loss. No further loss was noted after 3 days.

Table VIII. Comparative Effect of Technical Chlordane and HCS 3260 Treatment on Alfalfa Weevil Control and on Alfalfa Yields^a

Treatment and rate	Larvae numbers (A) and damaging rating (B) at indicated days after treatment ^b						Yields, lb dry weight ^c
	3	7		14		21	
	A	A	B	A	B	A	B
1.0 lb/acre							
Chlordane	5.7	9.0	2.7	143.0	4.3	552.6	6.0
HCS 3260	2.3	3.3	3.0	130.0	2.7	383.3	5.3
2.0 lb/acre							
Chlordane	4.3	3.0	2.8	117.3	3.7	366.6	5.0
HCS 3260	4.4	1.3	2.5	75.3	2.3	265.3	4.3
Control	64.7	140.3	3.7	398.0	7.0	650.0	8.0

^a HCS 3260, a high purity chlordane containing 95+ % α - and γ -chlordane. Applications of EC formulations made May 4, 1970, Lexington, Kentucky. All data are averages of three field replications. ^b Larvae per ten sweeps. Damage rating based on a 1-9 scale where 1 indicates no damage and 9 indicates complete skeletonization. ^c Averages of 10 ft \times 20 ft plots from each replication. Yield data collected 21 days after treatment. Treatment means followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.

in May and in September (Tables IV and V). In fact, the differences observed after the two different treatments were no greater than that variation experienced with the different replications within the same experiment. The data indicated that 21-day fresh cut alfalfa contained 2-3 ppm of the assayed residues when sprayed with 1 lb/acre of technical chlordane and 5-8 ppm when treated with the purified chlordane, HCS 3260. Based on the second test (Table V), this represents a 94% reduction in residues from the time of insecticide application (1 hr, fresh cut) until the time of harvest 21 days later (21 day, fresh cut). That the losses did not involve the selective dissipation of either α - or γ -chlordane, or that the "impurities" in technical chlordane did not appreciably effect the residual nature of these isomers is demonstrated by the data in Table VI. Ratios of the two isomers in the EC formulations of the insecticides compared very favorably with the same ratios observed on the treated alfalfa. With the HCS 3260 treated plants, the residues on the 21-day samples were composed entirely of α - and γ -chlordane. Small amounts of heptachlor epoxide were detected on comparable samples of the technical chlordane-treated alfalfa; occasionally, traces of a compound with a retention time the same as that of heptachlor also were observed.

Significant losses of insecticide residues occurred when the alfalfa plants were allowed to weather in the field for 3 days. There were reductions in all detected components of the residues and, while there was considerable variation, there did not appear to be a great selective loss of a particular residue during the process. By averaging the amount of loss by all samples in the first test and those in the second, it was found that weathering for 3 days reduced the level of residues by 56% in the May experiment and by 58% in the September test.

Volatilization of Residues. Fresh cut alfalfa containing α - and γ -chlordane residues (Table V, 1 lb/acre, HCS 3260) lost about 50% of those residues when let stand for 3 days at room temperature (Table VII). Thereafter, the rate of dissipation proceeded at a very slow rate, with a total loss in 14 days of 60 to 70%. The very rapid loss during the first 3 days corresponded to the loss of moisture from the samples. Although this indicated that the reduction of chlordane residues involved the loss of water, further experimentation showed that water loss was not required in order to get a substantial decline in the level of these residues on alfalfa. It was found, for example, that oven-dried alfalfa continued to lose residues even though there was no further decrease in moisture content of the plant material. Analysis of a hexane trap demonstrated that α - and γ -chlordane were volatilized intact from the alfalfa.

Biological Activity. Field evaluations of both technical chlordane-treated and HCS 3260-treated alfalfa showed that the purified chlordane was equal to or better than the technical chlordane for alfalfa weevil control (Table VIII). At comparable rates, the number of alfalfa weevil larvae collected from the plots was lower when HCS 3260 was used. Consequently, the degree of insect damage to the plants was slightly less. The final criterion of comparative effectiveness, that of alfalfa yield, was indiscernible. Both chlordane sprays yielded significantly more plant material than plots which were not treated, yet any real advantage of one material over the other was not demonstrated.

The improvement in the synthesis of technical chlordane to yield a product (HCS 3260) that was 98+ % α - and γ -chlordane serves as an excellent example of what can and should be done with a number of compounds currently considered commercial successes. Impurities in a pesticide, or a multi-component product, complicate the toxicological evaluations of a product and may result in the misinterpretation of those data which are obtained. This could create unnecessary health hazards and/or force an otherwise desirable product from the market. It is advisable, then, that the purity of all pesticides be critically evaluated and that the significance of any impurity in the biological activity of the material be clearly defined. With chlordane this has been largely accomplished. Over 98% of the applied AI of HCS 3260 consists of two known products which may be quantitated by common glc residue methods. Now, the fate of the high-purity chlordane when applied under practical conditions can be critically evaluated. This was virtually impossible with technical chlordane since about 65% of the AI was undefined as to chemical identity and consisted of five or more compounds.

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Residues of Organochlorine Insecticides and Their Metabolites in Soils in the Atlantic Provinces of Canada

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The persistence and breakdown of chlorinated insecticides in soil samples collected in the Atlantic Provinces of Canada were studied. Soxhlet extraction of the soil samples was achieved using hexane-2-propanol, 3 to 1. The 2-propanol was removed and the hexane layer purified using a Florisil column. The nature of the insecticide residues and their metabolites was studied using electron-capture gas chromatography with two column types. Thin-layer chromatography and chemical conversion to structurally related compounds confirmed the presence of some insecticides. The soil samples were taken from agri-

cultural lands in 1965 where organochlorine insecticides had been used. Forty-five per cent of all the soil samples investigated contained residues of DDT plus metabolites between 1 and 9 p.p.m. DDD and DDE were the chief metabolites of DDT. Thirty-two per cent of the total soil samples contained 0.75 p.p.m. of aldrin plus dieldrin. Heptachlor, heptachlor epoxide, and γ -chlordan were found in 9% of the soils analyzed in concentrations between 0.06 and 0.86 p.p.m. 1-Hydroxychlordene, a metabolite of heptachlor, was found in a small number of samples.

The persistence and breakdown of pesticides in soil under controlled conditions have been the subject of considerable study in recent years (1, 2, 11, 14, 18, 21). Residues of organochlorine insecticides in farm soils have been investigated by several authors (5, 10, 20, 21). The persistence and breakdown of insecticides in soils are related to a number of factors such as soil type and organic content, cultivation, rainfall, temperature, and soil microbial population (6). The conversion of aldrin and heptachlor to their epoxides (8) and DDT to DDE (21) in soil have been reported. Recently, Bowman, Schechter, and Carter (2) found that heptachlor was rapidly changed to 1-hydroxychlordene in dry soils with low organic content and that no conversion of heptachlor to its epoxide was detected in these soils. In another study, under field conditions, Bowman, Young, and Barthel detected a small amount of heptachlor epoxide and 1-hydroxychlordene in Norfolk fine sandy loam which had been exposed to heptachlor (3). Alexander (1) has indicated that many chemicals are resistant to degradation in the soil by microorganisms.

The objectives of this project were to establish quantitatively the extent to which residues of the organochlorine insecticides were occurring in Canadian Atlantic soils collected in Nova Scotia, New Brunswick, Newfoundland, and Prince Edward Island and the nature of the metabolites resulting from them.

Materials and Methods

Chemicals. The chemicals used were: DDDE, 1-chloro-2,2-bis(4-chlorophenyl)ethylene; γ -chlordan, 1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindan; and 1-hydroxychlordene, 4,5,6,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-4,7-methanoinden-1-ol.

Reagents. The following reagent grade solvents were distilled: acetone, *n*-hexane, dioxane, 2-propanol, and petroleum ether (30° to 60° C.).

Ethyl ether, U.S.P., was distilled, washed twice with water, and dried over anhydrous sodium sulfate, and a 2% v/v. anhydrous ethyl ether-alcohol solution was prepared therefrom.

Elution mixture 1, 120 ml. of ethyl ether was diluted to 1000 ml. with redistilled petroleum ether.

Elution mixture 2, 50 ml. of ethyl ether and 4 ml. of dioxane were diluted to 1000 ml. with petroleum ether.

Florisil commercially activated at 1200° F. was stored at 130° C. with three days' aging at room temperature in a stoppered bottle prior to use.

Silica gel-Camag DF-5.

Extraction mixture, consisted of *n*-hexane-2-propanol, 3 to 1.

Chromogenic agent, 1.7 grams of silver nitrate in 5 ml. of water and 10 ml. of 2-phenoxyethanol were diluted to 200 ml. with acetone (19).

Chlorine reagent, 0.3% chlorine in chloroform (9).

Hydrobromic acid reagent, 20 ml. of acetic anhydride was mixed with 10 ml. of 48% HBr in a flask which was cooled in ice and allowed to stand for 30 minutes before use.

Apparatus. GAS CHROMATOGRAPHY. The analytical instrument employed was a Wilkens Aerograph Hi-Fi Model 600-C equipped with an electron-capture detector containing a 250-mc. tritium ionization source, operated at 90 volts' potential across the detector. The recorder employed was a 1-mv. Sargent Model SR. The analytical column employed consisted of a 4.5-foot by 0.25-inch borosilicate glass tube packed with a 10% stationary phase which consisted of 4% G.E. methyl silicone plus 6% D.C. QF-1 (FS-1265) fluorosilicone on 60- to 80-mesh, acid-washed Chromosorb W. Before use, the column was conditioned for three days at 225° C. under a nitrogen pressure of 10 p.s.i.a. The operating parameters were: column temperature, 190° C.; detector temperature, 190° C.; injector flash heater, 220° C.; carrier gas, prepurified nitrogen; flow rate, 200 ml. per minute; range, 10; attenuator, 4. The injector of the gas chromatograph was equipped with a borosilicate glass liner.

The Dow 11 column consisted of 60- to 80-mesh, acid-washed Chromosorb W coated with Dow 11, 5% by

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weight. Before use, the column was conditioned for 2 days at 230° C. under a nitrogen pressure of 10 p.s.i.a.

The Dow Corning 200/QF-1 column consisted of 10% Dow Corning 200 and 15% QF-1 by weight on DMCS treated, acid-washed, 80- to 100-mesh Chromosorb W. The column was conditioned for 3 days at 230° C. under a pressure of 10 p.s.i.a.

Thin-layer chromatography-Desaga applicator.

Ultraviolet light source, General Electric, G15T8, 15-watt germicidal lamp.

Chromatographic columns, 20 mm. o.d., 16-mm. i.d. × 300 mm.

Soil Sampling. The sampling procedure was that described by Harris, Sans, and Miles (10). An area of approximately 5 acres was selected in a field and five subareas were sampled within this site. The subareas, 4 feet square, were placed diagonal to the field perimeter. Twenty-five 6-inch cores were taken from each subarea and cores from all five subareas were pooled in order to obtain a representative sample from the field. The pooled sample (approximately 10 pounds of soil) was sealed and stored in a refrigerated room at 8° C.

The samples were collected during the fall of 1965 and analyzed during the winter and summer of 1966.

Soil Types. The Atlantic soils analyzed comprised mineral soils including gravelly loam and also a complete range in soil texture from sandy to clay loam.

Preparation of Soil Samples. The soil samples were air dried at room temperature (22° C.). Large clay lumps were broken, and the sample was sieved using a 2-mm. sieve (U.S. series No. 10, 9 mesh). With the exception of the Newfoundland samples, a 50-gram soil portion was weighed and placed in a sintered glass thimble of a Soxhlet extractor. The Newfoundland samples varied from 32 to 50 grams. The extraction was accomplished with 150 ml. of hexane-2-propanol, 3 to 1, so that the thimble emptied once every 15 minutes. The sample was extracted for 4 hours. Soxhlet extraction using acetonitrile as the solvent gave lower recoveries of insecticides.

Purification Technique. The Soxhlet extract was transferred to a 1000-ml. separatory funnel and 100 ml. of petroleum ether was added. The mixture was shaken briefly, after which 500 ml. of distilled water and 10 ml. of saturated sodium chloride solution were added. The aqueous layer was discarded. The hexane-petroleum ether layer was further extracted with two 500-ml. portions of water to remove 2-propanol and then was dried over anhydrous sodium sulfate, and flash evaporated to 5 or 10 ml. The concentrate was chromatographed on a 4-inch Florisil column. The Florisil column was prepared by a slurry technique to avoid occluding air with the column. The column was first eluted with 75 ml. of elution mixture 1 which was collected separately as eluate 1 followed by 250 ml. of elution mixture 2 which was collected as eluate 2.

The elution system was a modified version of that described by Onley (16). The two fractions were concentrated to a small volume and diluted to 5 ml. with hexane. These solutions were subjected to quantitative and qualitative evaluation using gas chromatography.

Gas Chromatography. The SE-30/QF-1 liquid phase on Chromosorb W was satisfactory for a number of organochlorine compounds (12). The Dow 11 column was chosen as an alternate for the identification of insecticides during the early phase of the work. At a later phase of this study, a 10% Dow Corning 200, 15% QF-1 column was more satisfactory than the Dow 11 column. The use of this column was recently described by Burke and Holswade (4). An aliquot of 5 to 10 μ l. of the concentrated eluate was injected into the gas chromatograph. Reference chromatograms were prepared by injecting into the gas chromatograph a similar volume of a stock solution composed of the pure insecticides. For all quantitative studies, peak areas of known insecticide standards were compared with peak areas of unknown compounds. Standardization was carried out several times each day to check retention time and peak response for each insecticide. Care was taken to ensure that the response given by a particular insecticide was within the linear range.

Thin-Layer Chromatography. Thirty grams of silica gel was mixed with 70 ml. of water and stirred until a uniform slurry was obtained. A 0.2-mm. layer of the slurry was applied to a 20 × 20 cm. glass plate with the Desaga applicator. The coated plates were air dried and then further dried for one-half hour at 75° C. The plates were washed with absolute alcohol to remove materials which interfered at the development stage. The plates were activated at 130° C. for one-half hour and stored in a desiccator. An aliquot of eluate 1 or eluate 2 was then concentrated and spotted on the plate along with samples obtained from spiked blank soils. The chromatographic plate and developing tank were placed in a refrigerator at 4° C. and developed with hexane-ethyl acetate (9 to 1). The plate was removed from the tank, air-dried, sprayed with the chromogenic agent, and exposed to ultraviolet light for 5 minutes.

Chemical Confirmation. Eluate 1 which contained *p,p'*-DDT, and *o,p'*-DDT was refluxed for 30 minutes with a 5% solution of sodium hydroxide. These compounds were thereby converted to *p,p'*-DDE and *o,p'*-DDE, respectively. This solution was extracted with hexane, washed with water, and dried over anhydrous sodium sulfate.

With samples containing dieldrin, an aliquot of eluate 2 was taken and evaporated to dryness. One-half milliliter of HBr reagent was added to the residue, and the mixture was allowed to stand for 30 minutes at room temperature before use. Five milliliters of water, 3 ml. of hexane, and 1 ml. of saturated sodium sulfate solution were added to the residue, and the mixture was shaken. The hexane was separated, dried, and examined by gas-liquid chromatography.

With soil samples containing aldrin, an aliquot of eluate 1 was taken and evaporated to dryness. The residue was redissolved in 0.5 ml. of CHCl_3 and 0.1 ml. of chlorine reagent was added. After the solution stood for 5 minutes, the solvent was evaporated, and the residue was redissolved in 1 ml. of hexane and analyzed by gas chromatography.

Quantitative Recoveries. Quantitative recoveries of heptachlor, aldrin, heptachlor epoxide, *p,p'*-DDE, dieldrin, *o,p'*-DDT, DDD (rhotane), *p,p'*-DDT, γ -chlordan, and 1-hydroxychlordene were checked by adding these insecticides to air-dried soil samples at a concentration of 0.1 p.p.m. The insecticides were extracted from the soil samples and subjected to the column purification procedure. The recoveries of added insecticides and candidate metabolites ranged between 88 and 111%. The air-dried soil samples contained 3 to 5% moisture, and quantitative recovery calculations were based on the weight of these samples. No corrections were made for recoveries of insecticides found in agricultural soils based on the percentage recoveries of fortified samples at 0.1 p.p.m. Insecticide residue levels below 0.01 p.p.m. were reported as zero even when lower amounts of the insecticides were present.

Gas and Column Chromatography. Figure 1 shows a gas-liquid chromatogram of the standard insecticide mixture obtained by using the SE-30/QF-1 column. Two other columns, employing as liquid phases Dow 11 and Dow Corning 200/QF-1 on Chromosorb W, were found useful in separating and identifying insecticides and metabolites.

Elate 1 which was first eluted from the Florisil column contained heptachlor, aldrin, γ -chlordan, *p,p'*-DDE, *o,p'*-DDE, *o,p'*-DDT, DDD, and *p,p'*-DDT. Eluate 2 contained heptachlor epoxide, 1-hydroxychlordene, and dieldrin. On the SE-30/QF-1 column, heptachlor epoxide and γ -chlordan had approximately the same retention time. Since, however, heptachlor epoxide was found in eluate 2 and γ -chlor-

dan in eluate 1, no difficulty was encountered in estimating them. Similarly the pairs, dieldrin and *p,p'*-DDE, γ -chlordan and heptachlor epoxide could not be separated by the Dow 11 column alone, but could be distinguished because heptachlor epoxide and dieldrin appeared in eluate 2, while *p,p'*-DDE and γ -chlordan appeared in eluate 1.

Insecticide Residues in Agricultural Soils. Table I gives the levels of insecticides and metabolites found in soils along with the available history of insecticides used and crop rotation followed on the land from which the samples were obtained. The soil samples were taken from agricultural lands where insecticides had been used and where residues might be expected.

Forty-five per cent of all the soils investigated contained residues of DDT plus metabolites between 1 and 9 p.p.m. A single Nova Scotia orchid sample contained 20 p.p.m. and a single Newfoundland sample contained 17 p.p.m. Two to seven known applications of DDT had been used on these agricultural lands with a wide range of crops being grown.

The chief metabolites of DDT in the soils included DDE and DDD. The latter appeared chiefly in New Brunswick and Nova Scotia soil samples. DDE appeared in almost all soils containing DDT. The highest levels of DDE and DDD in the soils were 2.91 and 1.81 p.p.m., respectively. DDD has been reported to be a metabolite of DDT in lake water (13). Harris, Sans, and Miles (10) have also reported the presence of DDD in soils which had been exposed to DDT.

Thirty-two per cent of the soil samples analyzed contained 0.75 p.p.m. of aldrin plus dieldrin. The highest concentrations of aldrin and dieldrin were 2.5 and 4.04 p.p.m., respectively. These are slightly higher than those reported by Harris, Sans, and Miles for Ontario soils. The aldrin and dieldrin residues were found in soils where the common crops grown were rutabagas, potatoes, and vegetables.

Heptachlor occurred in significant amounts in five samples and heptachlor epoxide in six samples. The highest levels of heptachlor and heptachlor epoxide were 0.95 and 0.44 p.p.m. With two exceptions, γ -chlordan, present as a contaminant in technical heptachlor, occurred in significant amounts in all soils which contained heptachlor and heptachlor epoxide. γ -Chlordan was found in seven soil samples which had been exposed to heptachlor at levels between 0.06 and 0.86 p.p.m. In several Prince Edward Island soil samples exposed to heptachlor, a compound was detected on the SE-30/QF-1 column which had the same retention time as 1-hydroxychlordene. The presence of a compound having the same retention time as 1-hydroxychlordene was later confirmed using a 10% DC-200/QF-1 column. Four Prince Edward Island soils which had been subjected to frequent applications of heptachlor contained 1-hydroxychlordene in concentrations between 0.01 and 0.33 p.p.m.

A 15 to 20% constituent of technical DDT (7), *o,p'*-DDT, was found consistently in DDT-treated soils. The highest concentration of *o,p'*-DDT found in any soil sample was 2.63 p.p.m.

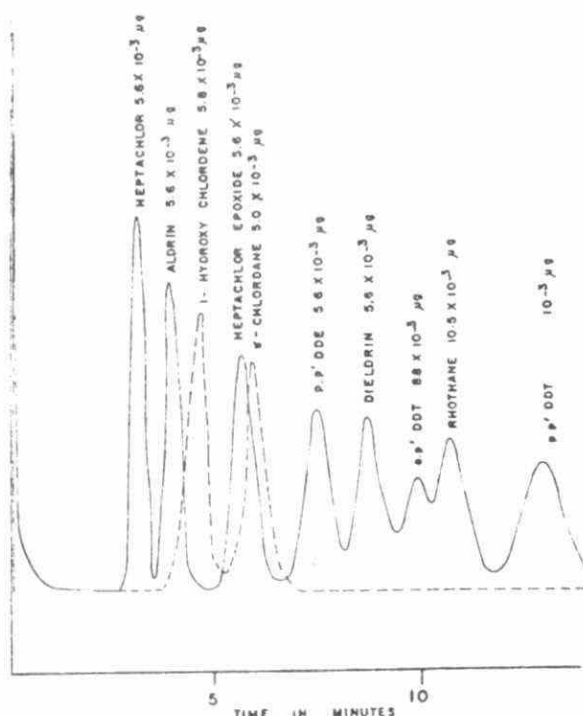


Figure 1. Separation of insecticides by means of a SE-30/QF-1 column

Table I. Soil Samples, Insecticide Residue Analyses, 1965

Cropping and Insecticide History				Residues in Soil Samples, P.P.M.										
Sample	Crops	Insecticide	Years of application	Total toxicant, lb./acre	Hept.	Hept. epox.	γ -Chlor.	Aldrin	Dieldrin	DDT		DDE	DDD	1-OH-C ^a
Prince Edward Island														
1	Rutabagas	Aldrin	'63	12	0	0	0	0.73	0.75	0.44	0.11	0.05	0.62	0
	Potatoes	DDT	'61	3										
2	Rutabagas	Heptachlor	Alternated use for	?	0.09	0.01	0.17	0.61	0.30	0.27	0.07	0.09	0	0.03
	Potatoes	Aldrin, DDT	7 years											
3	Vegetables	Heptachlor	Alternated use for	?	0.95	0.44	0.86	0.09	0.36	3.49	0.63	0.23	0	0.33
		Aldrin, DDT	15 years											
4	Rutabagas	Heptachlor	'59	5	0.05	0.07	0.06	0	0	0.72	0.12	0.15	0	0
	Strawberries	DDT	'60, '61, '62	4										
5	Vegetables	Heptachlor	Alternated use for	?	0.26	0.08	0.27	0.02	0	0.86	0.21	0.15	0	0.01
		Aldrin, DDT	many years											
6	Potatoes	DDT	'51, '54, '57, '60, '63	10	0	0	0	0	0	1.17	0.16	0.12	0	0
7	Orchard	DDT	'50-'63	20	0	0	0	0	0	0.06	0	0.03	0	0
	(under trees)													
8	Orchard	DDT	'50-'63	20	0	0	0	0	0	0.08	0.01	0.03	0	0
	(between trees)													
9	Strawberries	DDT	'58-'64	6	0	0	0	0	0.02	0.71	0.15	0.12	0.10	0
10	Potatoes	DDT	'59-'64	7.5	0	0	0	0	0	0.01	0.04	0.03	0	0
11	Rutabagas	Aldrin	'64	5	0	0	0	0	0	0.02	0	0.01	0	0
12	Rutabagas	Heptachlor	'57-'60	10+	0.68	0.05	0.48	0.59	0.33	0	0	0	0	0.08
	Carrots	Aldrin	'60, '65	8+										
13	Carrots	Aldrin	'60, '61, '62, '63, '64		0	0	0	0.44	0.86	0.08	0.01	0	0	0
Nova Scotia														
1	Orchard	DDT	'57-'61	4.5	0	0	0	0	0	0.66	0.10	0.20	0	
	(between trees)													
2	Orchard	DDT	'57-'61	4.5	0	0	0	0	0	1.37	0.22	0.42	0	
	(under trees)													
3	Carrots	Aldrin	'59, '61, '62, '65	30	0	0	0	2.13	4.04	0.15	0.05	0.02	0.03	
4	Vegetables	Aldrin	'65	6	0	0	0	0.49	0.17	0.01	0	0	0	
		DDT	'65	0.75										
5	Strawberries	DDT	'65	1	0	0	0	0.01	0	0.12	0.02	0.01	0	
6	Vegetables	Aldrin	'55-'65	12	0	0	0	0.39	0.38	0.43	0.10	0.05	0	
		DDT	'65	3										
7	Apple orchid	DDT	'55, '58, '59, '64	25	0	0	0	0.02	0.04	3.00	0.44	0.91	0.33	
• 1-Hydroxychlordece.														

^a 1-Hydroxychlorflorene.

Table I. Continued

Cropping and Insecticide History					Residues in Soil Samples, P.P.M.									
Sample	Crops	Insecticide	Years of application	Total toxicant, lb./acre	Hept.	Hept. Epox.	γ -Chlor.	Aldrin	Dieldrin	DDT		DDE	DDD	1-OH-C ^a
										<i>p,p'</i>	<i>o,p'</i>			
Nova Scotia														
8	Rutabagas	Aldrin	'65	5	0	0	0	1.03	0.18	0	0	0	0	
9	Vegetables	Aldrin	'59, '60, '63, '65	30	0.11	0	0.23	2.17	1.14	3.80	0.55	0.61	0	
		DDT	'60, '63, '64	10										
10	Carrots	Aldrin	'61, '63, '65	15	0	0	0	0.37	0.21	0.30	0.06	0.05	0.05	
11	Potatoes	Aldrin	'63, '64, '65	5	0	0	0	0.01	0	0.54	0.09	0.07	0	
		DDT	'63, '64, '65	6										
12	Potatoes	Aldrin	'61	4	0	0	0	0.28	0.30	0.75	0.14	0.12	0.20	
		DDT	'57, '65	3										
13	Vegetables	Aldrin	'60-'65	10	0	0	0	0.99	0.38	0	0	0	0	
14	Vegetables	Aldrin	'57, '60, '65	11	0	0	0	0.67	0.60	1.11	0.26	0.12	0.25	
		DDT	'64	3										
15	Orchard (between trees)	?	?	0	0	0	0	0	1.29	1.43	0.49	1.09	
16	Orchard (under trees)	?	?	0	0	0	0.09	0.52	1.42	0.70	1.47	0.52	
17	Orchard (under trees)	?	?	0	0	0	0	0.11	13.82	1.54	2.91	1.81	
18	Orchard (between trees)	?	?	0.02	0	0	0.15	0.16	0.46	0.23	0.28	0.07	
New Brunswick														
1	Orchard (under trees)	DDT	'62, '63	6	0	0	0	0	0.05	0.80	0.35	0.29	0.29	
2	Orchard (between trees)	DDT	'62, '63	6	0	0	0	0.01	0.08	0.70	0.19	0.19	0.26	
3	Orchard (under trees)	DDT	'57, '59	3-6	0	0	0	0	0	2.56	0.29	0.23	0.23	
4	Orchard (between trees)	DDT	'57, '59	3-6	0	0	0	0.01	0	0.48	0.07	0.08	0.05	
5	Corn	DDT	'57, '58, '59	3-6	0	0	0	0	0.04	0.28	0.07	0.06	0.17	
		DDD	'57	3										
6	Strawberries	DDT	'64	1	0	0	0	0	0	1.06	0.41	0.24	0.35	
7	Potatoes	DDT	'48-'65	45	0	0	0	0	0	2.08	0.52	0.29	0	

Table I. Continued

Cropping and Insecticide History					Residues in Soil Samples, P.P.M.									
Sample	Crops	Insecticide	Years of application	Total toxicant, lb./acre	Hept.	Hept. Epox.	γ -Chlor.	Aldrin	Dieldrin	DDT		DDE	DDD	1-OH-C
New Brunswick														
8	Potatoes	Aldrin	'63	2	0	0	0	0.09	0.14	0.23	0.03	0.02	0	
		DDT	'63, '64	6										
9	Potatoes	DDT	'53, '56, '59, '62	12	0.15	0	0	0.06	0.01	4.24	1.20	1.74	1.21	
10	Broccoli	Aldrin	'62, '65	0.5	0	0	0	0.14	0.02	0.18	0.13	0.05	0	
		Endrin	'62, '65	0.5										
11	Potatoes	DDT	'49-'63	20-40	0	0	0	1.42	0.06	0.50	0.13	0.07	0.16	
12	Brussel sprouts	Aldrin	'65	3	0	0	0	0	0	0.75	0.31	0.19	0.25	
13	Rutabagas	Heptachlor	'57	1.25	0.01	0	0	0.07	0.11	0	0	0	0	
14	Rutabagas	Aldrin	'65	15	0.01	0.05	0.22	0.01	0	0	0	0	0	
15	Cabbage	Aldrin	'61	4	0	0	0	0.02	0	0.49	0.09	0.05	0.05	
	Corn	DDT	'64	1										
Newfoundland														
1	Potatoes	Aldrin	'64	2.5	0	0	0	0.48	0.15	0.03	0	0.11	0	
		DDT	'64	2.5										
2	Root crop	Aldrin	'63	6	0	0	0	0.28	0.52	0.97	0.18	0.07	0	
3	Root crop	Aldrin	'61, '63, '65	7.5	0	0	0	1.39	0.68	13.84	2.63	0.65	0	
4	Cabbage	Aldrin	'60, '61, '65	24	0	0	0	2.50	0	5.41	0.97	0.28	0	
	Lettuce	DDT	'60, '61, '63, '64, '65	5										
5	Cabbage	Aldrin	'55, '58, '62, '64	10	0	0	0	0.40	0.36	0.84	0.18	0.05	0	
		DDT	'55, '58, '62, '64	5										
6	Root crop	Aldrin	3 of 4 years-'65	45	0	0	0	1.50	1.35	1.12	0.24	0.07	0	
		DDT	3 of 4 years-'65	15										
7	Turnips	Aldrin	'62, '63	5	0	0	0	0.29	1.45	0.41	0.09	0.02	0	
	Cabbage	DDT	'62, '63	3										
8	Root crop	Aldrin	'59, '60, '61, '62	20	0	0	0	0.13	0.08	1.40	0.26	0.03	0	
		DDT	'59-'65	7										
9	Cabbage	DDT	'63, '64, '65	6.75	0	0	0	0.03	0	1.56	0.34	0.03	0	
10	Root crop	Aldrin	'62, '63	7.5	0	0	0	0	0	0.34	0.08	0.03	0	
		DDT	'62, '63	3										

Chemical Conversion Results. Chromatograms were obtained from soil samples containing *p,p'*-DDT and *o,p'*-DDT before and after treatment with sodium hydroxide. These compounds were converted to *p,p'*-DDE and *o,p'*-DDE by sodium hydroxide, and the peaks obtained after treatment of soil samples containing them had the same retention time as pure authentic samples of *p,p'*-DDE and *o,p'*-DDE. Aldrin, dieldrin, heptachlor, and heptachlor epoxide do not react with sodium hydroxide.

Figure 2 shows the chromatogram of eluate 2 containing dieldrin, the same eluate after treatment with HBr, and a reference sample of pure dieldrin treated with the same reagent. Under the reaction conditions employed, dieldrin was converted to two compounds, which had longer retention times on the SE-30/QF-1 column than dieldrin. No attempts were made to identify these peaks. O'Donnell, Johnston, and Weiss (15) have shown that on treatment with hydrobromic acid-acetic anhydride at 120° C., dieldrin is converted to 6-acetoxy-7-bromo-6,7-dihydroaldrin. Hamence, Hall, and Caverly (9) report that only one compound was detected under the above reaction conditions when gas chromatography was employed as the analytical tool.

Soil samples containing aldrin were treated with the chlorine reagent and gave two peaks on the SE-30/QF-1 column which had identical retention times as pure aldrin treated with the same reagent. Chlorine adds to the unchlorinated double bond of aldrin to form the *trans*-dichloride (17). Under the same reaction conditions, chlorine did not add to the double bond in *p,p'*-DDE.

Figure 3 shows the separation of several insecticides and related materials found in soil samples in eluates

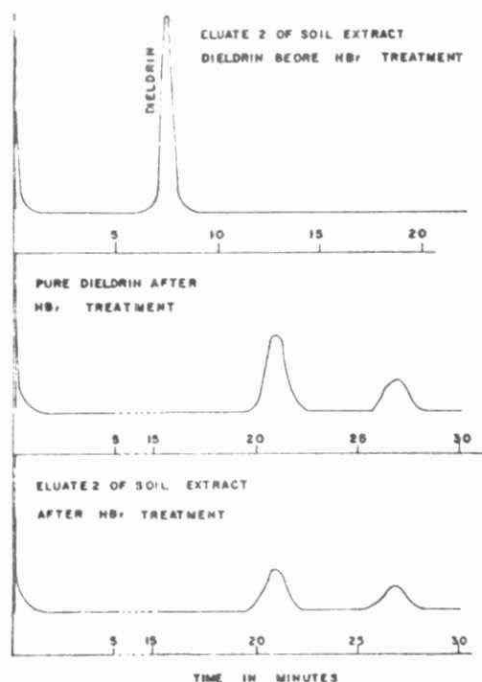


Figure 2. Treatment of dieldrin with HBr along with a soil sample containing dieldrin before and after treatment

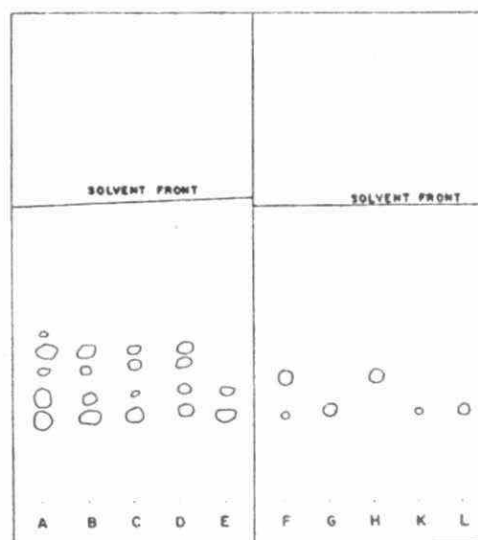


Figure 3. Thin-layer chromatography

A. Standard solution of insecticides (top to bottom) aldrin, *p,p'*-DDE, heptachlor, *o,p'*-DDT, *p,p'*-DDT.

B, C, D, E. Eluate 1 of soil samples containing several insecticides.

F. Standard mixture of dieldrin and heptachlor epoxide.

G, H, K, L. Eluate 2 of soil samples containing heptachlor epoxide and dieldrin

1 and 2 using thin-layer chromatography. The behavior of reference materials is presented also. Better separation and less diffusion of the spots occurred when the chromatographic tanks and solvent were kept at 4° C. The quantity of insecticide in the soil samples was estimated visually after development by comparing the spot size and intensity with known standards of the pure insecticides chromatographed on the same plate. The semiquantitative estimations of the amounts of insecticides present in the sample using thin-layer chromatography was in general agreement with the results obtained by gas-liquid chromatography. The solvent system hexane-ethyl acetate (9 to 1) did not move 1-hydroxychlordehene on a thin-layer plate. However, a developing system consisting of hexane-ethyl acetate (7 to 3) separated 1-hydroxychlordehene from dieldrin and heptachlor epoxide.

The results reported in this paper indicate that significant amounts of insecticides may be present in agricultural soils where organochlorine compounds have been frequently applied. This study was exploratory in nature and the data are not statistically representative of the Atlantic Provinces. Insecticides can be absorbed from soils by certain plants, and it is important to know the insecticide levels in selected soil samples in order to prevent contamination of crops by insecticides.

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II. INVITATION PAPERS

INSECTICIDE POLLUTION AND SOIL ORGANISMS^{1,2}

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Abstract

Information available at present indicates that although residues of some organochlorine insecticides are accumulating in soils, they are not generally resulting in serious deleterious effects on soil microorganisms or soil animals. Some insect pests have become resistant to the cyclodiene insecticides, and also, population shifts have occurred, resulting in serious insect control problems. Plants are absorbing residues of some organochlorine insecticides from soils. While this does not appear to be a direct hazard to human health, the problem is potentially more serious with crops used for animal feed. Because of magnification, minute residues in crops may result in unacceptable residue levels in milk and animal products. The most serious problem is that of general environmental contamination with certain organochlorine insecticides and the side effects which may occur. Because of these side effects, the organochlorine insecticides are being rapidly replaced with organophosphorus and carbamate compounds, partly because of a popular misconception that these newer materials are less persistent. Data are presented to show that this is not necessarily the case. While advocating restrictions on the use of the organochlorine insecticides, the author suggests caution in haphazardly replacing them with materials which may have even more serious environmental side effects.

Although we hear a great deal about environmental pollution these days, the major emphasis is being placed on air and water pollution. Few people realize that there is a third aspect which is receiving less attention, i.e. pollution of the soil. Although we must have air to breathe and water to drink, it is from the soil that, directly or indirectly, we produce most of our food. The top few inches of the soil contain vast numbers of beneficial living organisms, which contribute to the structure, formation, and fertility of the soil. Without them the soil could conceivably become so unproductive that we would be unable to feed the exploding population of the world. Unfortunately, not all soil organisms are beneficial. Coleopterous, dipterous, and lepidopterous insects are serious crop pests, as are some other arthropods such as millipedes, mites and symphylans. Nematodes are a serious pest; fungi and bacteria cause diseases in plants; and weeds are an important problem. Consequently, modern agriculture injects large quantities of chemical pesticides into the soil to control these pests. What effects do these pesticides have on the soil organisms and their environment?

Although not entirely justified, much of the current concern over pesticide pollution of our environment is centred around insecticides. Consequently, this is

¹ Invitation paper presented as part of a symposium on "Pollution and the Entomologist" at the Annual Meetings of the Entomological Societies of Ontario and Canada, Guelph, Ontario, August 26-29, 1969

² Contribution No. 436, Research Institute, Canada Department of Agriculture, London, Ontario

the area of pesticide pollution about which we know the most, and to which I will restrict this discussion. However, I would like to suggest that many of the comments I make pertaining to insecticides may apply equally well to nematocides, fungicides or herbicides.

Before discussing the effect of insecticide residues in soils on soil organisms, it would perhaps be of value to review a little of the history of the use of insecticides in Canada and, using southwestern Ontario as an example, to illustrate the residue buildup in soil which has occurred.

Prior to 1945, insecticides in general use were inorganic compounds, of which lead arsenate was the prime example. This material was used extensively for many years, and is still used to some extent. Arsenic is highly persistent in soil and, as would be expected, residues have accumulated. Table I summarizes results obtained by Miles (1968) in a survey which he conducted on a number

TABLE I. Arsenic residues¹ in soils in southwestern Ontario in relation to crop grown

Crop	Average residue (ppm ²)	Lowest — Highest
sugar beets	3.7	1.5 — 8.6
corn	3.9	1.1 — 8.5
cereals	3.9	1.3 — 6.9
forage and pasture	4.0	2.9 — 5.5
tobacco	4.7	1.4 — 6.9
greenhouse vegetables	5.1	2.3 — 6.9
vegetables	5.4	1.1 — 26.6
orchards	53.5	10.2 — 121.0

¹ Arsenic occurs naturally in soils usually at < 10 ppm

² ppm calculated on the basis of the oven-dry weight of soil

of farms in southwestern Ontario. Arsenic occurs in soils naturally, usually at concentrations of less than 10 ppm. Taking this natural arsenic content into consideration, it is apparent that most of our agricultural soils in Ontario do not contain significant levels of arsenic. However, in vegetable soils, arsenic residues were as high as 26.6 ppm, while in orchard soils, the residue levels ranged from 10.2 to 121 ppm.

DDT, the first synthetic organic insecticide, was introduced about 1945, and was followed in a few years by the cyclodiene insecticides, of which aldrin, dieldrin, heptachlor, and endrin are common examples. Between 1945 and 1965 these materials were used extensively for insect control. Their use has now declined drastically. DDT is highly persistent in soil, but is slowly degraded, primarily to DDE. Aldrin and heptachlor are only moderately persistent in soil, but are degraded in part to the persistent epoxides, dieldrin and heptachlor epoxide. Endrin, a stereoisomer of dieldrin, is also persistent in soil. In 1964, we undertook a survey of the residue levels of organochlorine insecticides occurring in farm soils in southwestern Ontario. Residues were present in virtually all soils (Table II). The highest residue levels of DDT were found in tobacco, vegetable, and orchard soils. Residues of the cyclodiene insecticides, primarily dieldrin, were present in most soils with the exception of sugar beet soils, and orchard soils. Vegetable soils contained the highest residues of the cyclodiene insecticides. This study has been continued on a reduced scale on 16 farms. Between 1964 and 1966, residues of aldrin, dieldrin, and DDT increased while residues of endrin decreased (Table III). Five-year samples will be taken on these same farms in 1969. It is to be hoped that some levelling off of the aldrin, dieldrin, and DDT levels will occur to match the decreased use of the organochlorine insecticides which is said to have occurred since 1965.

TABLE II. Organochlorine insecticide residues in soils in southwestern Ontario in relation to crop grown

Crop	DDT and related materials (ppm ¹)	Cyclodiene insecticides and related materials (ppm)	Total residue (ppm)
sugar beets	0.4	0	0.4
forage and pasture	0.5	0.3	0.8
corn	1.2	0.2	1.4
cereals	1.4	0.4	1.8
greenhouse vegetables	1.5	0.8	2.3
tobacco	3.2	0.6	3.8
vegetables	9.5	1.6	11.1
orchards	61.8	0	61.8

¹ ppm based on oven-dry weight of soil

TABLE III. Average levels of organochlorine insecticide residues found in the soil on 16 farms in southwestern Ontario

Year	Organochlorine insecticide residues in soil (ppm ¹)			
	aldrin	dieldrin	endrin	DDT
1964	0.25	0.48	0.22	17.4
1966 ²	0.47	0.78	0.12	23.9

¹ ppm based on oven-dry weight of soil

² Further samples to be taken in September 1969

As a result of the development of cyclodiene insecticide resistance by some species of soil insects, and the persistence of some of the organochlorine insecticides in soil and other sectors of the environment, there has been a general trend away from these materials to other synthetic organics, primarily the organophosphorus insecticides and, to a lesser extent, the carbamates. The earliest organophosphorus and carbamate insecticides, such as parathion, malathion, diazinon, and carbaryl, were much less persistent in soil than the organochlorine insecticides. In our survey studies, we seldom find residues of these materials. Unfortunately, the limited persistence of these early substitutes has led to the development of a popular misconception that they are generally less persistent than the organochlorine insecticides. In fact, this is not necessarily the case. In our laboratory, we screen all experimental insecticides under controlled conditions to determine their persistence in soil, and the materials are classified into three groups: slightly residual; moderately residual; and highly residual. Over the past three years we have screened about 100 insecticides in this fashion. There are a number of short residual materials whose biological activity disappears in a matter of 2 to 4 weeks (Figure 1). However, there is a second generation of materials, many of which are now registered or close to being registered for use, which can be classed as moderately residual, i.e. they are similar in their persistence in soil to aldrin (Figure 2). At the rates at which they are recommended for use, which are often 2 to 7 times that required with aldrin, I have no hesitation in predicting that, in some instances, there will be a carry-over of residues of these materials in the soil from one year to the next. Even more disturbing is the fact that we have found some experimental insecticides to be *as persistent as dieldrin* (Figure 3), and at least one of these materials is being actively developed

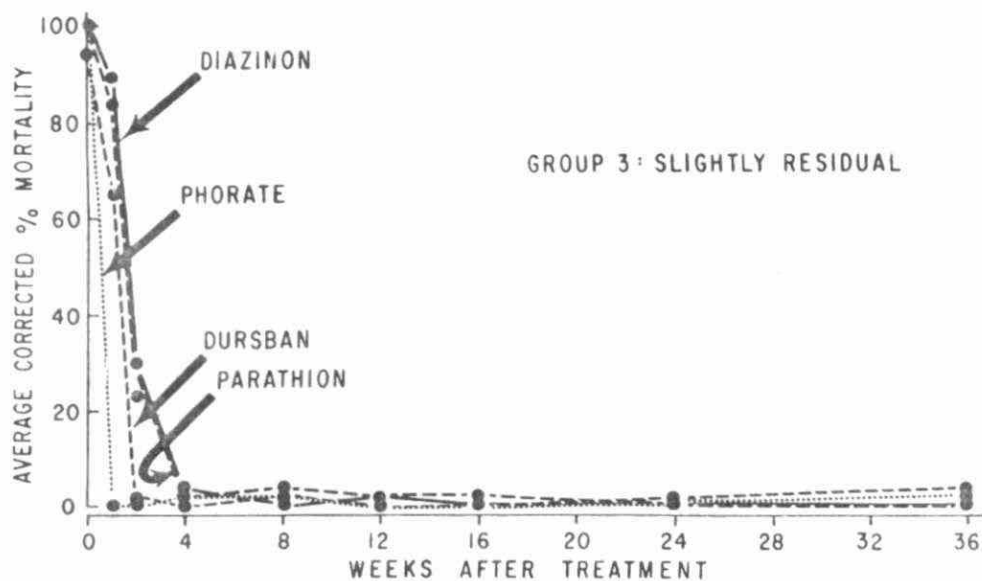


FIGURE 1. Persistence of biological activity of some insecticides in a sandy loam (Laboratory Studies)

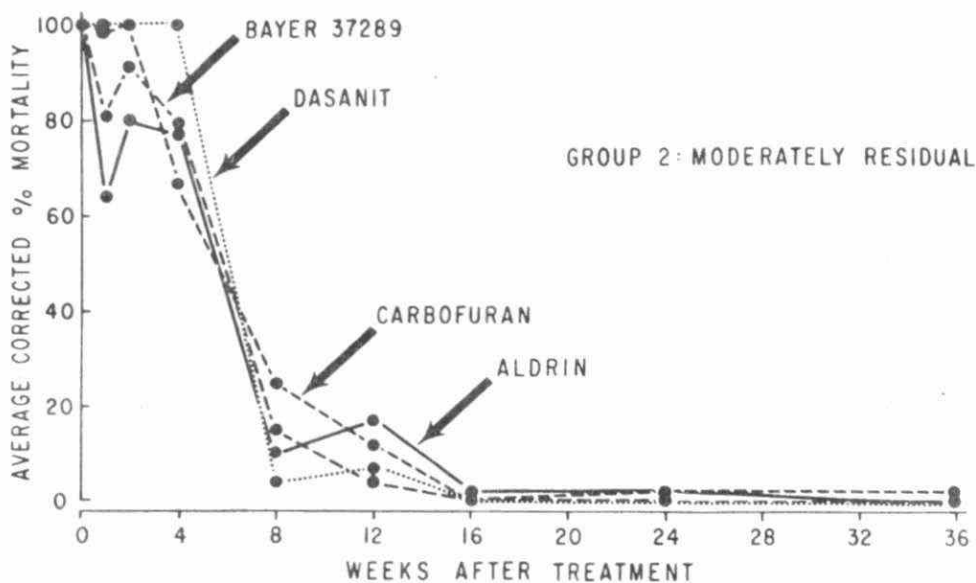


FIGURE 2. Persistence of biological activity of some insecticides in a sandy loam (Laboratory Studies)

for soil insect control. Thus, in our panic to move away from the persistent organochlorine insecticides, we are on the point of recommending materials

which are more hazardous to use, and may in themselves be as persistent as, or more persistent than, materials such as aldrin and heptachlor which have already been banned for use.

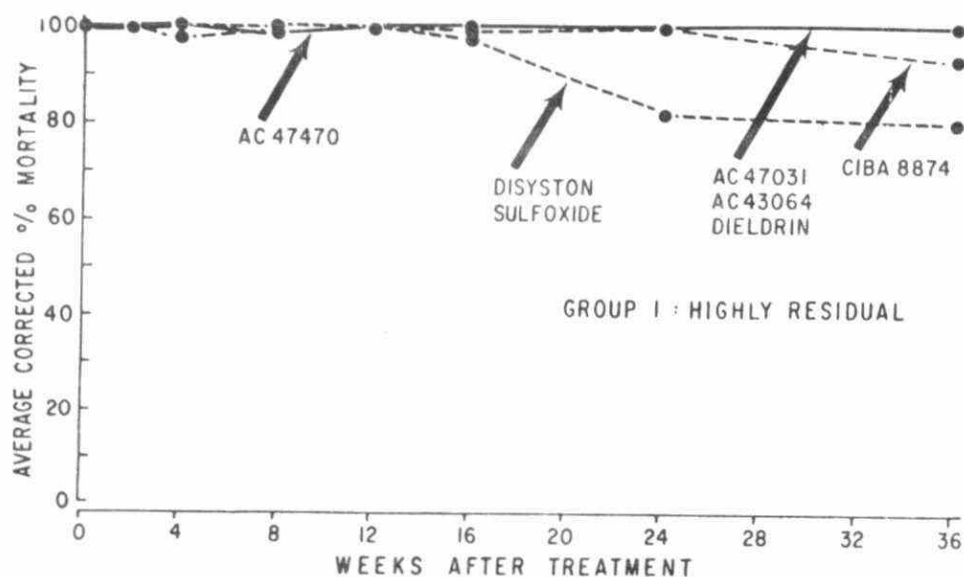


FIGURE 3. Persistence of biological activity of some insecticides in a sandy loam (Laboratory Studies)

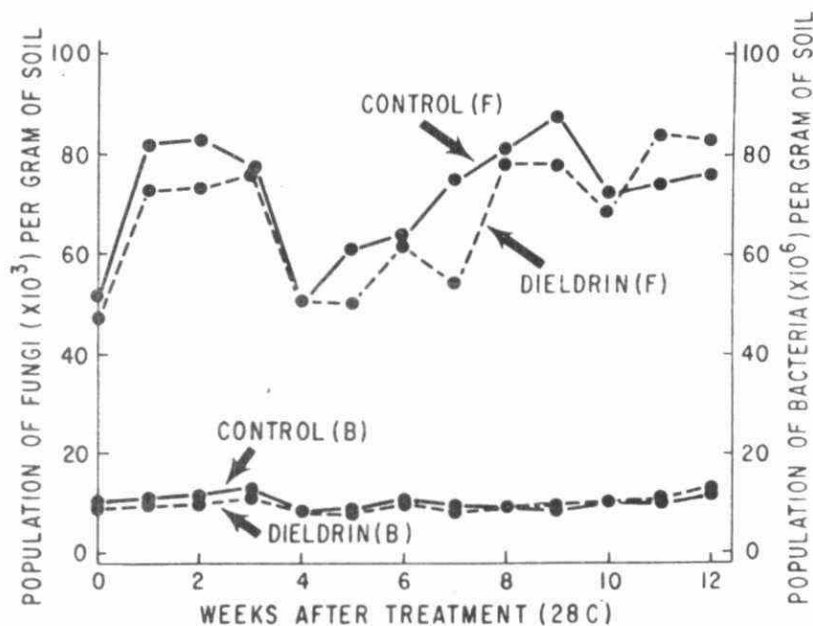


FIGURE 4. Influence of dieldrin (2000 ppm) on soil microorganisms

What effect then will these insecticide residues in soil have on the soil organisms?

Soil microorganisms are of primary importance to soil fertility. During the past three years, Dr. C. M. Tu at our laboratory has been studying the effect of insecticides on soil microorganisms. The effect of dieldrin on total counts of fungi and bacteria in soil is illustrated in Figure 4. This work was done at the excessively high concentration of 2,000 ppm, since the primary purpose of the experiment was to isolate a highly tolerant species capable of degrading dieldrin. However, dieldrin, even at this high concentration, had little effect on the total population of fungi and in fact, after 10 weeks, the population had adapted so well that the counts in the dieldrin treatment exceeded those in the control. Similar results were obtained with bacteria. Tu also obtained the same type of result with aldrin, and others have obtained similar results with heptachlor, heptachlor epoxide, and DDT. Thus it would appear that the organochlorine insecticides have little effect on soil microorganisms. Recently, Tu has been examining the effect of organophosphorus insecticides on soil microorganisms. Results (Figure 5)

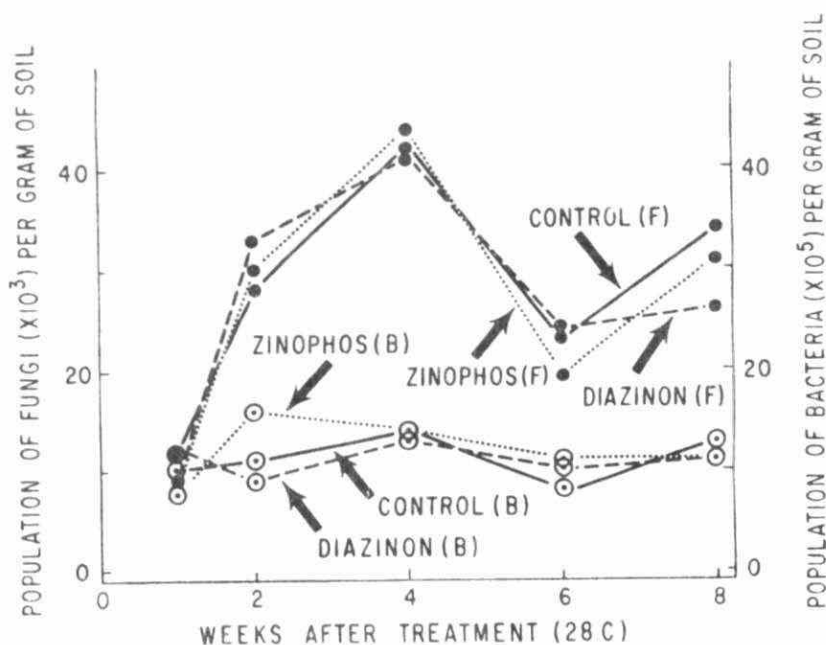


FIGURE 5. Influence of diazinon and Zinophos (100 ppm) on soil microorganisms

obtained with two organophosphorus insecticides, diazinon and Zinophos, both of which are commonly used for soil insect control in Canada, indicated that neither material at 100 ppm had any pronounced effect on either the fungal or bacterial populations in the soil. Tu has obtained similar results with several other experimental organophosphorus and carbamate insecticides which are being actively developed for soil insect control. Generally speaking, it would appear that the synthetic organic insecticides have little detrimental effect on soil microbial activities.

Although they have little effect on soil microorganisms, what effects do the microorganisms have on the insecticides? The two major cyclodiene insecticides

used in Ontario were aldrin and heptachlor, but the predominant cyclodiene insecticide residues in our soils are dieldrin and heptachlor epoxide. The explanation of this phenomenon is simple — the microorganisms convert aldrin to dieldrin and heptachlor to its epoxide. Recently in our laboratory, we isolated 92 species of soil microorganisms from a sample of soil taken from a farm in southwestern Ontario known to have had an extensive history of aldrin treatment. In *in vitro* experiments conducted over a period of six weeks, we found that 89 of the 92 species (97%) were able to convert aldrin to dieldrin in amounts ranging from a trace to 9.2% of the added aldrin, (Tu *et al.*, 1968). The results obtained with two species of microorganisms are illustrated in Figure 6. In the one case, the conversion

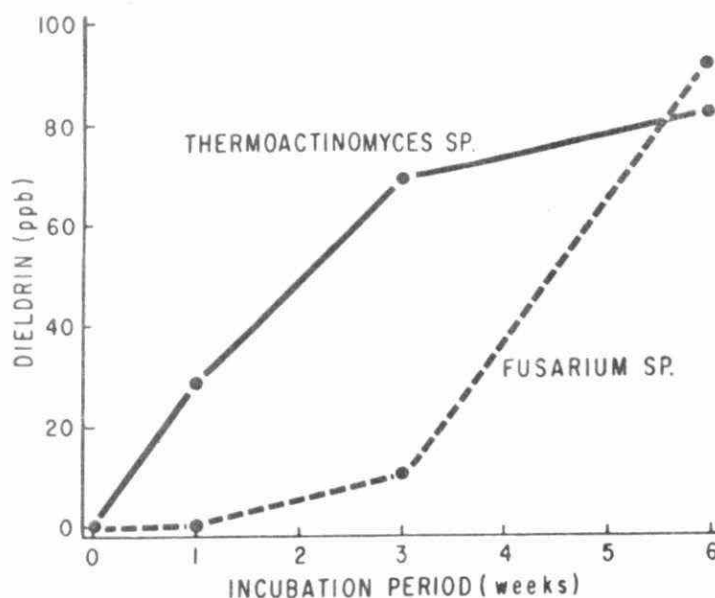


FIGURE 6. *In vitro* microbial conversion of aldrin to dieldrin

of aldrin to dieldrin began almost immediately. In the other instance, a period of adaptation was required before the organism could metabolize the aldrin. Both bacteria and fungi were capable of the conversion. However, the fungi appeared to be the most active converters. These results, then, explain the rapid disappearance of aldrin residues in soils, i.e. perhaps 90% of it will vaporize, while the remainder is oxidized by soil microorganisms to produce dieldrin.

Subsequent studies also pointed up another important difference between aldrin and dieldrin. Some soil microorganisms were capable of degrading dieldrin. Figure 7 illustrates the results obtained with two species capable of converting aldrin to dieldrin and, subsequently, degrading dieldrin, possibly to non-toxic hydrophylic metabolites. However, only 22 of 92 species (24%) of the soil microorganisms, primarily bacteria and actinomycetes, showed any ability to degrade dieldrin. Thus, the conclusion is obvious — so long as there are residues of aldrin in the soil, the microbial conversion of aldrin to dieldrin will be greater than the microbial degradation of dieldrin, and thus dieldrin residues will tend to accumulate in soil.

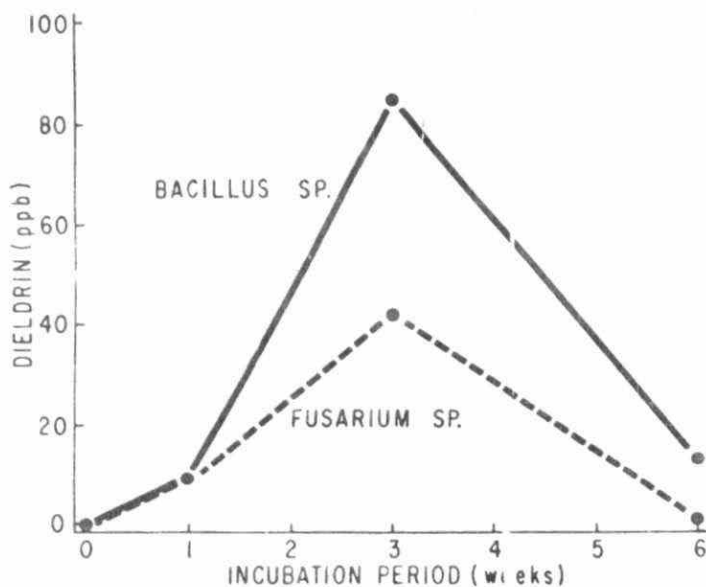


FIGURE 7. *In vitro* microbial conversion of aldrin to dieldrin and subsequent degradation of dieldrin

More recently we have been looking at the degradation of heptachlor by soil microorganisms and have obtained some extremely interesting and important results (Miles *et al.*, 1970). The *in vitro* microbial and chemical degradation of heptachlor is complex (Figure 8). Heptachlor is volatile and a large part of it in water or soil will vaporize. In addition, we determined that microorganisms convert heptachlor to its epoxide and identified those involved. During the experiment we also found that a limited number of microorganisms are capable of degrading heptachlor to chlordene. And, we discovered a major pathway of chemical and microbial degradation of heptachlor to much less toxic metabolites. This involves the hydrolysis of heptachlor to 1-hydroxy-chlordene which is, in turn, metabolized by microorganisms to 1-hydroxy-2,3-epoxy-chlordene. This latter is then metabolized by microorganisms to an unknown compound, possibly keto-chlordene. From the pollution point of view, these results are of considerable importance. The hydrolysis to 1-hydroxy-chlordene in aqueous solution occurs quite rapidly. Thus, it would appear that it is highly unlikely that heptachlor could become a serious pollutant of water. In soils, the picture is not quite so clear. While we have evidence that 1-hydroxy-chlordene is found in soils, the amount is about equal to the amount of heptachlor epoxide formed. At present we are trying to establish quantitatively the importance of the various pathways of degradation of heptachlor in soil, soil-water, and water systems.

Microorganisms also degrade DDT to DDE and some evidence is now being accumulated that indicates that they are active at some stage in the degradation of organophosphorus and carbamate insecticides. It is becoming apparent that soil microorganisms have a major role in the degradation of insecticidal residues in soil.

What are the effects of insecticides on soil animals? Soil animals belong primarily to three invertebrate phyla, the Protozoa, Annelida, and Arthropoda. Of

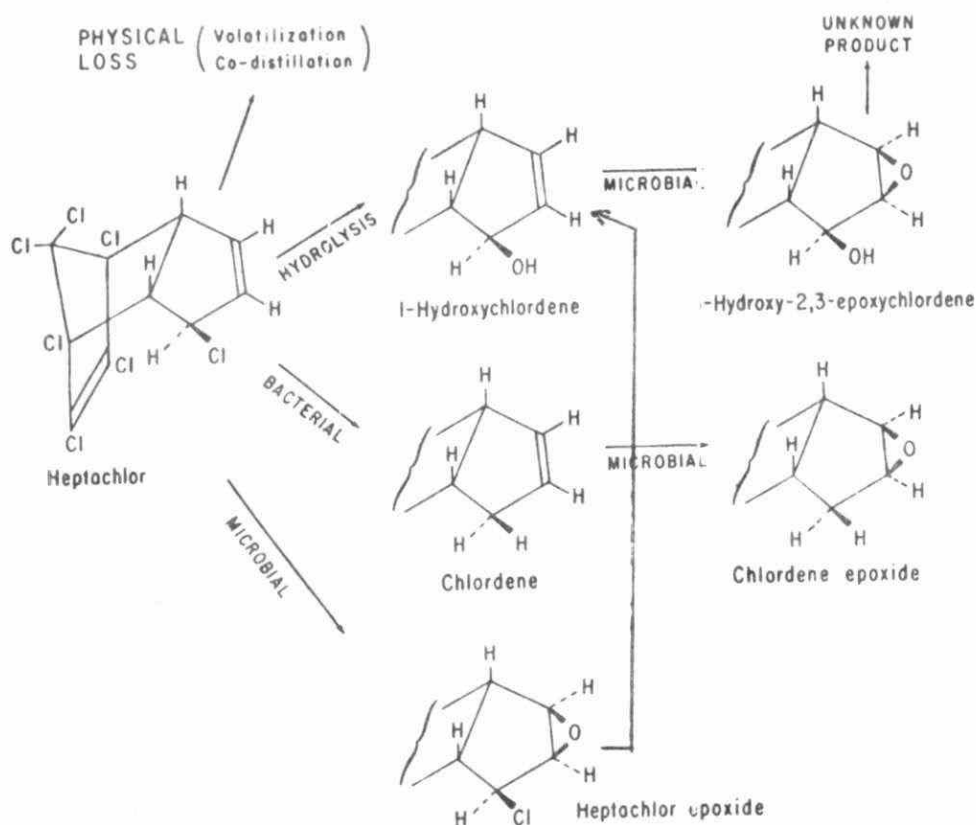


FIGURE 8. Scheme for chemical-microbial degradation of heptachlor

these, little is known of the effects of pesticides on the soil protozoans. Fortunately, some excellent work is being carried out by Dr. C. A. Edwards and his co-workers at Rothamstead on the effects of pesticide residues in soil on other soil invertebrates, and in a recent paper Edwards (1969) summarized the results of several years of research.

Earthworms are of major importance in soil since they break down much of the plant debris reaching the soil, and turn over the soil and aerate it. Thus, pesticide residues in soils which appreciably reduce numbers of earthworms could constitute a serious problem. Edwards has shown that earthworms are tolerant to many pesticides. None of the chlorinated hydrocarbons affected them seriously, other than heptachlor and chlordane. Among the common organophosphorus and carbamate insecticides, only phorate and carbaryl were particularly toxic. Other organophosphorus insecticides did reduce earthworm populations, but they recovered relatively quickly since the chemicals were all short-residual compounds. However, it is difficult to predict what will happen to earthworm populations with the second generation of organophosphorus and carbamate insecticides now being developed for use. These materials, which are moderately persistent in soil, may have a more lasting effect. I do think that it is significant, however, that the materials which have had the most toxic effect on earthworms, i.e. chlordane, phorate, and carbaryl, are three materials that are commonly being substituted in place of the organochlorine insecticides that are being phased out.

An equally important aspect of the earthworm problem is that they can concentrate the organochlorine insecticides in their adipose tissue. Edwards has found aldrin and dieldrin residues in the bodies of earthworms at concentrations $10 \times$ that of the surrounding soil. Similar results have been obtained with heptachlor, heptachlor epoxide, and DDT. Such uptake of these persistent insecticides constitutes a serious problem, since earthworms serve as an important source of food for birds, which in turn may concentrate insecticides to an even greater degree. Hence, earthworms, as a lower link in the food chain, are an important source of undesirable chemical residues in higher animals. Fortunately, Edwards has found that the less residual organophosphorus insecticides are not concentrated to any great extent in earthworms. More information is required on the second generation organophosphorus and carbamate insecticides now being developed.

Edwards and his group have concentrated much of their effort on soil arthropods. Many soil arthropods are beneficial in that they contribute to the disintegration and digestion of plant residues, and to the breakdown of debris into its organic and inorganic constituents. Examples include the wood lice, millipedes, oribatid mites, and several species of insects, particularly springtails and larvae of beetles and flies. Other arthropods, including millipedes, mites, symphylans, and lepidopterous, coleopterous and dipterous larvae are serious pests. Still others, including spiders, centipedes, and parasitic mites are predatory on both the beneficial and pest species of soil insects. Recently Edwards (1969) summarized results of an interesting study on the effect of DDT and aldrin applied at normal rates of soil application on soil arthropods. As would be expected, both pesticides radically altered the number of individuals within the population of each species of soil arthropod, and also the number of species present in the soil. The overall effects obtained are illustrated in Figure 9. DDT resulted in a slight decrease in total arthropod weight, while aldrin resulted in a drastic decrease. When predator weights were compared, both DDT and aldrin drastically reduced the soil predators. However, when the beneficial arthropod weights were compared, DDT resulted in an increase over the control, while aldrin resulted in a sharp decrease. Edwards showed that the increase in beneficial arthropod weight obtained with DDT was because DDT was toxic to predatory mites, and as a result a pronounced increase in Collembola occurred. Since Collembola are of major importance in the degradation of plant debris to organic matter, it is apparent that DDT, in this instance, has a beneficial influence. As would be expected, both aldrin and, to a lesser extent, DDT, resulted in a drastic decrease in pest weight. The results with aldrin would seem to indicate that, in contrast to DDT, it has a pronounced deleterious effect on both predators and beneficial soil arthropods, as well as on the arthropod pests. However, this particular series of experiments was conducted on soils which were not cultivated. When Edwards ran parallel experiments with control and aldrin plots on cultivated and uncultivated soil, he found that cultivation alone reduced the soil arthropods to an extent similar to that obtained with aldrin. He concluded that the change produced by aldrin was not significant in terms of soil fertility. The general conclusion which he has reached from his studies is that it is only in forest and woodland soils that insecticide pollutants are a potential hazard, in that they may slow down the processes of soil formation and maintenance of soil fertility. He suggests that aerial spraying with large quantities of pollutants in forests should be eliminated wherever possible.

As shown in Figure 9, both aldrin and DDT markedly reduce arthropod pest populations. The most effective materials against the common insect pests in soil were aldrin and the other cyclodiene insecticides. As a result these materials were used extensively across Canada and the United States, and, as shown earlier (Tables II and III) cyclodiene insecticide residues have built up to significant levels in soils,

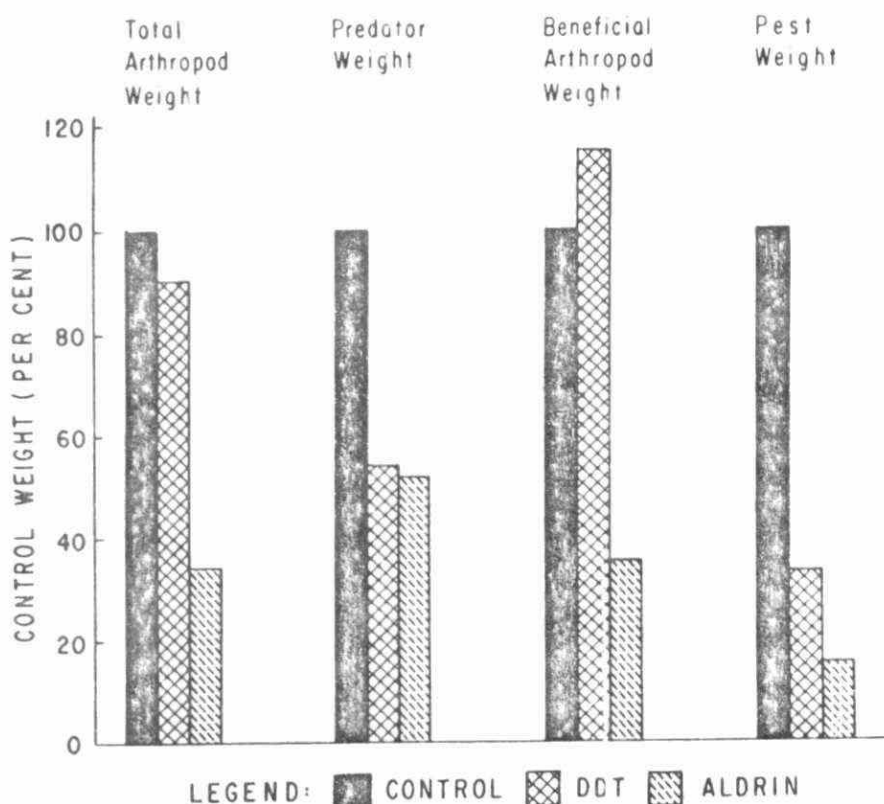


FIGURE 9. Influence of DDT and aldrin on soil arthropod populations [Modified from C. A. Edwards (1969)]

particularly tobacco and vegetable soils. In many instances, these residue levels were sufficiently high to result in constant selection pressure on soil insect populations. Consequently, within a relatively short period of time some soil insects developed a high degree of cyclodiene insecticide resistance. In Canada this was particularly the case with the root maggots (Table IV). Five species developed

TABLE IV. Degree of cyclodiene insecticide resistance to aldrin in various species of root maggots in southwestern Ontario

Species	Resistance level
bean seed fly (seed maggot)	× 255
onion maggot	× 591
seed-corn maggot (seed maggot)	× 770
cabbage maggot	× 1,127
carrot rust fly	× 5,600

a very high level of resistance to aldrin, ranging from × 255 with the bean seed fly to × 5600 with the carrot rust fly. In addition, cross-resistance occurred to all the other cyclodiene insecticides, but not to DDT or the organophosphorus

or carbamate insecticides. In the United States, wireworms and the corn rootworm have also become resistant to the cyclodiene insecticides. The use of these materials has also resulted in species shifts which have resulted in serious economic problems. A good example of this is the cutworm problem in tobacco in southwestern Ontario. In the 1950's the major cutworm species in tobacco were the black (*Agrotis ipsilon* Hufnagel), and variegated (*Peridroma saucia* Hübner) cutworms. The dark-sided cutworm (*Euxoa messoria* Harris) was a pest of minor importance. By 1957, aldrin and heptachlor were being used extensively for cutworm control in tobacco. These materials were remarkably effective against the black and variegated cutworms (Table V), and when applied at the recommended rate of 1½ pounds per acre, provided 100% control. Within 2 to 3 years both species had ceased to be a problem. However, the dark-sided cutworm was highly tolerant to aldrin (Table V) and the other cyclodiene insecticides, and as the

TABLE V. Toxicity of aldrin EC applied as a surface application to moist sandy loam to 3-4 instar larvae of the variegated, black and dark-sided cutworm

Species	Average corrected % mortality at indicated rate of application (pounds active ingredient per acre)				
	¼	½	1	2	4
variegated	94	100	100	100	100
black	45	95	100	100	100
dark-sided	0	0	0	0	0

two susceptible species were eliminated, the dark-sided cutworm moved in to fill the vacuum. By 1961, the outbreak reached extremely serious proportions, and it was not until 1966 that an adequate method of control was worked out utilizing DDT. An intensive research program is now under way to find adequate replacements for DDT.

To date, there has been no development of resistance to the organophosphorus insecticides by soil insects in Canada although the corn rootworm has become resistant to diazinon in the United States. The main criteria for the development of organophosphorus insecticide resistance is the same as that which was necessary with the cyclodiene insecticides, i.e. that residues in soil should be sufficiently high to result in constant selection pressure on the population. So long as we use short residual organophosphorus insecticides such as diazinon, it is unlikely that this will occur, other than with insects which have only one generation per year. However, in some instances, these short residual materials have not provided effective pest control. As a result we are beginning to switch to more residual organophosphorus and carbamate insecticides (Figure 2). These materials will undoubtedly result in higher selection pressure, and, in 5 to 6 years, I would expect that we will begin to see the development of organophosphorus insecticide resistance in certain species of soil insects, particularly the cabbage maggot. In addition, we may also encounter population shifts similar to those I described with cutworms. The substitute materials which we are developing to replace DDT for cutworm control are short residual materials. While they will be effective against the dark-sided cutworm, they will not necessarily be effective against the black and variegated cutworms. Within 4 to 5 years after their introduction, we may find that the dark-sided cutworm is no longer a problem, while the black and variegated cutworms are ascendant.

In any discussion of soil pollution we cannot ignore the effects of pollutants on the plants we grow in the soil. The most obvious effect is phytotoxicity. With insecticides this has happened in some instances where attempts have been made

to convert orchards to other types of farming. Arsenic at high concentrations, as occurs in orchards, can be phytotoxic to some crops. A less obvious but more important effect is that residues are absorbed from soils by some crops. Root crops are often quoted as the prime examples of this phenomenon. Table VI shows

TABLE VI. Residues of organochlorine insecticides in a clay loam soil and residues found in root crops grown in this soil

Soil-Crop	Organochlorine insecticide residues (ppm ¹)		
	DDT	aldrin	dieldrin
Soil — before planting	0.36	0.53	0.88
— after harvest	0.34	0.48	1.08
Carrots	T ²	0.02	0.11
Radishes	T	T	0.05
Turnips	0	0	0.03
Onions	0	0	0.02

¹ ppm calculated on oven-dry weight of soil and fresh weight of crop

² T = trace = < 0.01 ppm

residues of DDT, aldrin, and dieldrin absorbed by root crops grown in a clay loam soil contaminated with these three insecticides. The amounts of DDT absorbed by crops are generally insignificant. Contrary to popular opinion aldrin is not absorbed by crops to any significant extent, or if it is, it is rapidly converted to dieldrin. Significant dieldrin residues appear in root crops, particularly carrots (Table 6). However, the indications are that residues absorbed by crops used for human consumption are generally well below the established tolerances.

More serious is the problem of absorption of some insecticide residues from soil by crops used for animal feed (Table VII). Residues of DDT and aldrin did

TABLE VII. Residues of organochlorine insecticides in a clay loam soil and residues found in crops used for animal feed

Soil-Crop	Organochlorine insecticide residues (ppm ¹)		
	DDT	aldrin	dieldrin
Soil — before planting	0.39	0.37	1.02
— after harvest	0.43	0.14	1.19
Sugar beets (roots)	0.01	0	0.07
Carrots	0	0	0.04
Potatoes	0	0	0.03
Sugar beets (tops)	0.03	0	0.03
Corn	0.04	0	0.02
Oats	0.03	0	0.02
Alfalfa	0.01	0	0.02

¹ ppm calculated on oven-dry weight of soil and fresh weight of crop

not appear in what would be considered significant amounts. However, virtually all the crops tested absorbed dieldrin to some extent. Sugar beets absorbed the greatest amounts, followed by carrots, potatoes, sugar-beet tops, corn, oats, and alfalfa. Since animals tend to concentrate dieldrin in their fatty tissues, this constitutes a potentially serious problem. There is little agreement as to the concentration of dieldrin in crops which will result in unacceptable residue levels in milk and other animal products. Obviously, this will depend on a number of factors

including the dieldrin concentration in the crop, the proportion of the crop in the animal diet, and the length of time that the crop in question is fed to the animals. It is often assumed that levels as low as 0.02 ppm of dieldrin in a crop would result in unacceptable residue levels in milk and animal products. On this basis, which is admittedly rather arbitrary, it would appear that in a number of instances in our experiments, residues of dieldrin in the soil were sufficiently high to result in significant residue levels in crops grown for animal feed. However, I should point out that we were working with soils in which the cyclodiene insecticide residues were abnormally high, and that, as a general rule, most of our agricultural soils contain less than one-third that amount of residue.

I am often asked, "What level of insecticide residue in the soil constitutes pollution?" This is an extremely difficult question to answer. Unfortunately, many people tend to take the results of chemical analysis *per se* and make a judgment on this basis. This is the wrong approach. Table VIII shows results we

TABLE VIII. Influence of organic content of soil on the biological activity of dieldrin and its absorption by carrots

Soil type	% O.M.	Dieldrin (ppm ¹)		Bioactivity ³
		in soil	in carrots ²	
sandy loam	1.0	1.77	.20	97
clay loam	6.1	1.91	.04	6
clay loam	29.4	1.76	.01	2
muck	41.7	2.00	.01	0
muck	70.0	1.99	.01	0

¹ ppm based on oven-dry weight of soil and fresh weight of crop

² tolerance = 0.1 ppm

³ test insect = first instar crickets

obtained when we grew carrots in soils containing approximately the same concentration of dieldrin (2 ppm) but varying amounts of organic matter. As you can see, both the insecticidal activity assessed by bioassay and absorption of the residues by crops decreased in proportion to the amount of organic matter in the soil. Thus, a residue of 2 ppm of dieldrin in a sandy loam would constitute a fairly serious level of pollution. By contrast, 2 ppm of dieldrin in a muck soil is of little consequence. Most of our high insecticide residues, incidentally, occur in muck soil where they are inactivated. We seldom find a mineral soil where residues of dieldrin exceed 0.5 ppm or, at the very most, 1 ppm. We have obtained similar results with both heptachlor epoxide and DDT in that activity and/or absorption by crops is in proportion to the amount of organic matter in the soil.

To sum up: The information which we have to date, which deals primarily with the organochlorine insecticides, indicates that the present residue levels in soil are generally not resulting in any serious deleterious effects on beneficial soil organisms. The organochlorine insecticides do not effect soil microorganisms and hence are not altering soil fertility; with one or two exceptions, they are not causing permanent deleterious effects on beneficial soil animals. On the other hand, some insect pests have become resistant to the cyclodiene insecticides, and in other instances, population shifts have occurred, resulting in serious insect control problems. Also, some plants are absorbing residues of some cyclodiene insecticides from soil. While these residues are not at serious levels in crops for human consumption, the problem is potentially more serious in crops used for animal consumption.

At present there is a trend away from the persistent organochlorine insecticides to organophosphorus and carbamate insecticides, on the questionable as-

sumption that these materials will have less serious side effects. Unfortunately, this trend to the newer materials is developing into a stampede which, in the end, could result in even more serious problems than we have at the present time. I say this for a number of reasons. First, as I pointed out, there is a popular misconception that the newer materials are less persistent in soil, and this is not necessarily the case. Secondly, while most organochlorine insecticides are almost insoluble in water and do not move vertically downward in soil to any great extent, many of the newer materials are slightly to moderately soluble so that, consequently, we run the risk of contaminating ground water, as well as streams and lakes. Third, these newer materials are usually more toxic to handle and can be as toxic, or more toxic, to beneficial soil animals, fish and wildlife. Fourth, while considerably more expensive, they do not provide the same degree of insect control as the organochlorine insecticides. By switching to them, we are deliberately decreasing crop yield or quality. Thus, I feel that the organochlorine insecticides still have an important place in some phases of insect control.

At the same time we cannot ignore the problem of environmental pollution. Although this problem has been blown up out of all proportion by the opponents of pesticides, there is sufficient information available to indicate that the persistent organochlorine insecticides are, indeed, a serious problem. The evidence, however, is that, in most instances, the problem is not coming so much from agricultural operations as from municipal uses, such as massive Dutch elm disease control programs and home owner use, and from rural programs, such as biting fly control in recreational areas. I strongly advocate that the use of the organochlorine insecticides should be banned for these purposes, and that their use in agricultural and medical entomology should be restricted to those situations where the use is absolutely essential and where it can be demonstrated that this use is not resulting in a serious environmental pollution problem. Such applications should be by permit only.

In conclusion, I would like to state quite bluntly that we cannot do without pesticides. We need them to produce, store, and process our food; to protect vast areas of forest; to protect man and his animals against disease-carrying insects; to treat imported food and fiber to prevent the introduction into Canada of undesirable species of foreign pests; and to provide control of nuisance pests, e.g. mosquitoes and black flies, so that man can enjoy his leisure time. In recent years there has been a great deal of publicity and discussion over non-pesticide methods of controlling pests, such as biological control. The simple fact is that, other than in isolated instances, present non-pesticide approaches are impractical, expensive or require much more research before they might be developed to a practical level. It appears that pesticides will be with us for a long time. Their assets greatly outweigh their liabilities. We must simply learn how to use them properly, and how to regulate and control their use.

Chemical Designations of Experimental Materials

American Cyanamid AC43064: cyclic ethylene (diethoxyphosphinothioyl) dithioimidocarbonate
 American Cyanamid AC47031: cyclic ethylene (diethoxyphosphinyl) dithioimidocarbonate
 American Cyanamid AC47470: cyclic propylene (diethoxyphosphinyl) dithioimidocarbonate
 Bayer 37289: 0-ethyl 0-2,4,5-trichlorophenyl ethylphosphonothioate
 Ciba 8874: 0-(2,5-dichloro-4-iodophenyl) 0,0-diethyl phosphorothioate
 Dasanit: 0,0-diethyl 0-p-(methylsulfinyl) phenyl phosphorothioate
 Disyston sulfoxide: 0,0-diethyl S-2-(ethylsulfinyl) ethyl phosphorodithioate
 Dursban: 0,0-diethyl 0-3,5,6-trichloro-2-pyridyl phosphorothioate

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Behavior of Heptachlor Epoxide in Soil¹

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ABSTRACT

A study, in both the laboratory and the field, was conducted to elucidate the factors influencing the behavior of heptachlor epoxide in soil. Laboratory studies were devoted to determining the factors influencing insecticidal activity. As a direct contact poison, the epoxide was as effective or more so than heptachlor against 24- to 48-hr-old crickets, *Gryllus pennsylvanicus* (Burmeister); picture-winged flies, *Chaetopsis debilis* (Loew); and black cutworms, *Agrotis ipsilon* (Hufnagel). However, in soil it was less effective than heptachlor. Tests indicated that in soil it vaporized to an extent sufficient to cause fumigant toxicity to insects and that volatility was dependent on soil type, moisture, and temperature. The major factor

influencing insecticidal activity was soil type, with toxicity in moist soil negatively correlated with organic content. Moisture and temperature were factors of lesser importance. Tests on persistence of biological activity in mineral soil indicated that the epoxide was more persistent than heptachlor, but slightly less persistent than dieldrin. Microplot field trials with 3 soil types containing 0.9, 16.6, and 51% organic matter treated with approximately 2 ppm of heptachlor epoxide indicated that while it was persistent in soil, insecticidal activity, absorption by crops, and mobility in soil were proportional to the organic content, rather than the concentration of insecticide.

Earlier papers have noted that the biological activity of insecticides in soil depends on numerous inter-related factors including the susceptibility and behavior of each species of soil insect, soil type, moisture, and temperature, and the persistence of biological activity (reviewed by Edwards 1966, Harris 1972a, b). It also has been pointed out (Harris 1972a) that, since each insecticide possesses its own specific properties, efficient methods of soil-insect control will be devised only after investigating in detail the behavior of each compound in relation to the factors noted above. Heptachlor has been used extensively throughout the world for soil-insect control, and considerable effort has been devoted to defining the parameters influencing its activity in soil (Bowman et al. 1965a; Burkhardt and Fairchild 1967; Harris 1966, 1967, 1971; Wiese 1961). It has been shown also that, in general, it is of limited persistence in soil and that it degrades, in part, to 1-hydroxychlordehene (Bowman et al. 1965a, Duffy and Wong 1967, Carter and Stringer 1970) and to heptachlor epoxide (Gannon and Bigger 1958). Miles et al. (1969, 1971) in *in vitro* experiments determined that heptachlor is rapidly hydrolyzed to 1-hydroxychlordehene in aqueous solution and that soil microorganisms can convert heptachlor to heptachlor epoxide and the latter to 1-hydroxychlordehene. The extent to which 1-hydroxychlordehene will persist in soil is not clear, but it does not appear to be insecticidally active (Bowman et al. 1965b, Harris 1972b). By contrast, heptachlor epoxide is persistent (Wilkinson et al. 1964) and insecticidally active in soil. It is therefore important to define the factors influencing its behavior in soil.

METHODS AND MATERIALS.—Procedures used, both in the laboratory and field have been described, in detail, in previous reports (Harris 1969, 1971, 1972a; Harris and Sans 1972) and will be given in less detail in this report.

Laboratory Studies.—Studies in the laboratory were on the factors influencing the biological activity of heptachlor epoxide in soil. Test insects included 24-

to 48-hr-old crickets, *Gryllus pennsylvanicus* (Burmeister); adult picture-winged flies, *Chaetopsis debilis* (Loew); and 3rd-stage larvae of the black cutworm, *Agrotis ipsilon* (Hufnagel). Unless otherwise specified, the tests were done utilizing crickets as the test insects. The heptachlor epoxide used was 99.4% pure. Tests were done to determine the toxicity of heptachlor epoxide to insects using direct-contact, residual-contact (glass surface), and soil applications. In soils, soil type, moisture, and temperature were taken into consideration. Tests were also done to determine its volatility and persistence of biological activity in soil.

Assessment of the insecticidal activity of heptachlor epoxide as a direct-contact insecticide was made using a Potter spray tower. Heptachlor was used as a standard insecticide to serve as a basis for comparison. Tests were conducted against crickets, flies, and cutworms. Following treatment the insects were placed in observation containers and held under controlled environmental conditions.

Residual contact toxicity was assessed by applying the insecticide, dissolved in distilled, chromatographed (2:1 activated charcoal:florisil) n-pentane, in microgram amounts to the bottom of a 9-cm glass petri dish. Following evaporation of the solvent, moisture and a source of food were provided, the test insects were introduced, and the dishes were covered and held under controlled environmental conditions. In all tests log dosage-probit lines were constructed.

Activity of the insecticide in soil was assessed by incorporating the insecticide, dissolved in distilled, chromatographed n-pentane into the soil and evaporating the solvent. The treated soils were weighed into containers, moisture and food were provided, the test insects were introduced, and the containers were covered and placed at the required environmental conditions. Experiments on the influence of soil type on insecticidal activity involved 6 soil types: a Plainfield sand, Beverly fine sandy loam, 2 samples of Bookston clay, and 2 samples of muck containing different levels of organic matter. Preliminary screening tests were done using field-moist and oven-dry samples of sand and a muck containing 70% organic matter. More detailed tests were conducted in soils at field-moisture capacity. All soils were slightly acidic to neutral. Organic content ranged from 0.5 to 70%. Other character-

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istics of the soils have been described (Harris 1966). The impact of soil moisture on biological activity of heptachlor epoxide was assessed using the fine sandy loam at 6 moisture levels: 0% (oven-dry), 1% (air-dry), 3.1, 6.2, 9.3, and 12.0% water. The effect of temperature on the biological activity of heptachlor epoxide in soil was determined using 2 soil types: the sandy loam containing 0 and 12% water, and a muck containing 70% organic matter and 163% water. In all tests the percent water was calculated on the basis of the oven-dry weight of the soil. Following treatment and prior to introduction of the test insects the soils were preconditioned to the required temperature levels of 15, 21, 27, and 33°C.

The toxicity of insecticide vapors emanating from soil treated with heptachlor epoxide was measured by placing the test insects on a gauze screen suspended 0.5 cm above the surface of the treated soil. Tests on the volatility of heptachlor epoxide were conducted in relation to soil temperature, moisture, and type.

Persistence of biological activity of heptachlor epoxide in soil was determined using procedures outlined elsewhere (Harris 1969). Heptachlor and dieldrin were used as standards for comparison. The soil type was a Plainfield sand. The insecticide applications applied were $2 \times$ the approximate LD_{50} , i.e., 0.8, 0.6, and 1.2 ppm for heptachlor epoxide, heptachlor, and dieldrin, respectively. Soils were brought up to field moisture capacity each week, and samples were removed at intervals up to 48 weeks. Tests were done under controlled environmental conditions.

All tests were conducted using environmental chambers programmed to provide the conditions required; i.e., $27 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH, and 24-hr photoperiod, unless otherwise specified. Direct-contact toxicity tests with heptachlor and heptachlor epoxide were done using 4 insecticide concentrations: 0.001, 0.01, 0.1, and 1.0% solution. Preliminary tests comparing their activity in soil were run using 5 insecticide concentrations: 0.1, 0.5, 1, 5, 10 ppm (based on oven-dry weight of soil). Duplicate groups of 10 insects were used at each concentration. Each test was repeated a 2nd time, and the results were averaged. Corrections for natural mortality were made (Abbott 1925). In more detailed studies, log dosage-probit lines were constructed using 5–8 insecticide concentrations causing mortalities ranging from approximately 15 to 90%. Duplicate groups of 10 insects were used at each concentration. Each assay was run 3 times and

Table 1.—Toxicity of heptachlor and heptachlor epoxide as contact insecticides.

Insect	Insecticide	Average corrected % mortality at indicated % insecticide solution			
		0.001	0.01	0.1	1.0
Cricket	heptachlor	0	0	100	100
	h. epoxide	0	75	100	100
Fly	heptachlor	0	100	100	100
	h. epoxide	0	100	100	100
Cutworm	heptachlor	0	0	11	100
	h. epoxide	0	0	20	100

results were pooled prior to statistical analysis of the dosage-mortality data by computer. Controls, using the solvent treatment only, were run with all tests. Results of the direct-contact toxicity tests are expressed in percent solution, residual contact toxicity in micrograms, and soil treatments in ppm.

Field Studies.—These were conducted using microplots, 0.9×2.2 m, enclosed with fiberglass barriers set into the soil to a depth of 20 cm. Six plots were required utilizing 3 "soil types": 2 plots each of Plainfield sand, muck, and a 1:1 (vol/vol) sand:muck mixture. Organic contents were 0.9, 51, and 16.6% respectively. The original soil in the microplots was removed to a depth of 20 cm and replaced with the test soils. Three of the plots were filled with the untreated sand, sand:muck, and muck, the remaining 3 plots with soil treated with heptachlor epoxide. The treatments were applied by spraying batches of each soil type with an EC formulation of heptachlor epoxide in a rotating cement mixer (Harris and Sans 1972). The level of treatment was approximately 2 ppm (based on oven-dry weight of soil). Following application of the insecticide the treated soils were placed immediately in the microplots. The soil was treated during the last week of May. "Long Nantes" carrots were planted, 2 rows/plot, immediately after treatment.

Soil samples were taken immediately before treatment, immediately after treatment, and at monthly intervals for 3 months. In addition, strata samples were taken in 5-cm sections to a depth of 30 cm after treatment and 4 months later. Samples of carrot roots and tops were taken for analysis at harvest.

Table 2.—Toxicity of heptachlor and heptachlor epoxide to 1st-stage cricket nymphs as soil insecticides.

Soil type	% water	Insecticide	Average corrected % mortality at indicated ppm insecticide in soil				
			0.1	0.5	1	5	10
Sandy loam	11.3	heptachlor	15	100	100	100	100
		h. epoxide	0	95	100	100	100
	0	heptachlor	0	0	15	100	100
		h. epoxide	0	0	0	100	100
Muck	163.0	heptachlor	0	0	0	100	100
		h. epoxide	0	0	0	0	65
	0	heptachlor	0	0	15	100	100
		h. epoxide	0	0	0	100	100

Table 3.—Influence of soil type on the biological activity of heptachlor epoxide.

Soil type	% organic matter	% water	LD ₅₀ (ppm)	95% C.I.
Sand	0.5	7.5	0.19	0.18–0.20
Fine sandy loam	1.0	12.3	.25	.24–.26
Clay	6.1	22.0	.46	.38–.55
	29.1	96.0	5.11	4.80–5.37
Muck	11.7	96.0	7.17	6.88–7.50
	70.0	110.0	9.89	9.47–10.28

The soil samples were split, with one part being used for chemical determinations of heptachlor epoxide residues, the remainder for bioassay of insecticidal activity. Details of techniques of extraction, cleanup, and analysis of the samples by GLC have been described (Harris and Sans 1972, Sans 1967). Residues are reported in ppm based on the oven-dry weight of the soil and fresh weight of the crop. Assays for biological activity of the heptachlor epoxide residues in the various soil types were done by exposing 24- to 48-hr-old crickets to the field-collected samples using the bioassay techniques and controlled laboratory conditions just described. Each assay was done in triplicate, and the experiment was repeated a 2nd time. Results of the 2 tests were averaged.

RESULTS AND DISCUSSION.—Laboratory Studies.—In tests comparing the toxicity of the epoxide to heptachlor as a direct-contact insecticide, the results (Table 1) indicated that the epoxide was as effective as heptachlor against flies and cutworms and more toxic to crickets. However, when incorporated into moist sandy loam the epoxide was ca. 1/2 as toxic to crickets as heptachlor (Table 2). It was also less effective in moist muck soil. Both materials, however, would be classed as effective soil insecticides. Other studies (Harris and Mazurek 1964) have shown that part of the effectiveness of aldrin and heptachlor in soil can be attributed to the fact that they are volatile and thus act as both contact and fumigant poisons. Tests on the volatility of heptachlor epoxide in soil indicated that in moist sandy loam it was sufficiently volatile to cause fumigant mortality, particularly as soil temperature increased. A concentration of 0.6 ppm heptachlor epoxide in moist sandy loam caused 0%

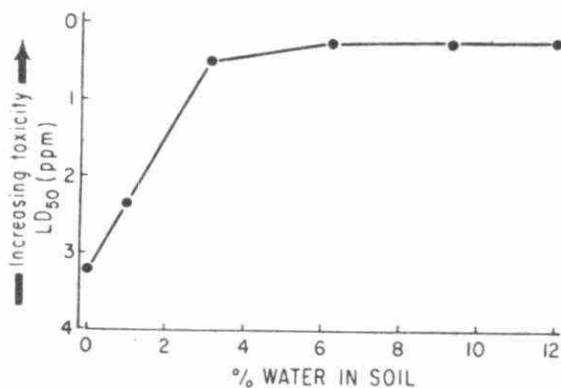


Fig. 1.—Influence of soil moisture on the biological activity of heptachlor epoxide in fine sandy loam.

Table 4.—Influence of temperature on the toxicity of heptachlor epoxide as direct contact and residual contact (glass surface) applications.

Method of application	Temp °C	LD ₅₀	95% C.I.
Direct contact ^a	15	0.012	0.011–0.013
	21	.011	.010–.012
	27	.011	.010–.012
	33	.010	.009–.011
Residual contact ^b	15	.160	.145–.176
	21	.083	.076–.090
	27	.082	.077–.086
	33	.080	.074–.086

^a LD₅₀ in % solution.

^b LD₅₀ in µg applied to the glass surface.

mortality at 15°, 57% at 21°, and 100% at 26 and 33°C. In addition to being temperature dependent, volatility was also related to soil moisture and type. In dry sandy loam it was necessary to increase the epoxide concentration by a factor of 58 to achieve equivalent fumigant toxicity to that obtained in the sandy loam with a concentration of 0.6 ppm. In moist muck, similar fumigant mortality was obtained at the various temperature levels by increasing the epoxide concentration by factor of 41. In its performance as a soil insecticide, the behavior of heptachlor epoxide more closely paralleled that of dieldrin than aldrin and heptachlor, i.e. both heptachlor epoxide and dieldrin were as or more effective than aldrin and heptachlor as contact poisons, but less effective in soil, probably because they are less volatile.

The preliminary screening tests indicated that soil type influenced the biological activity of heptachlor

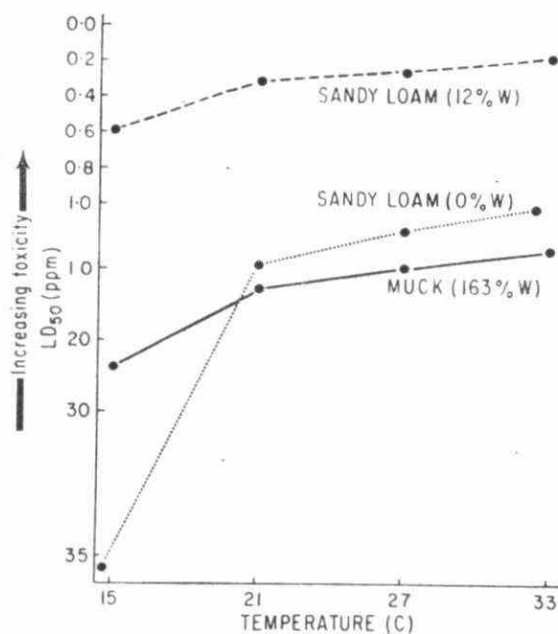


Fig. 2.—Influence of soil temperature on the biological activity of heptachlor epoxide in relation to soil moisture and type.

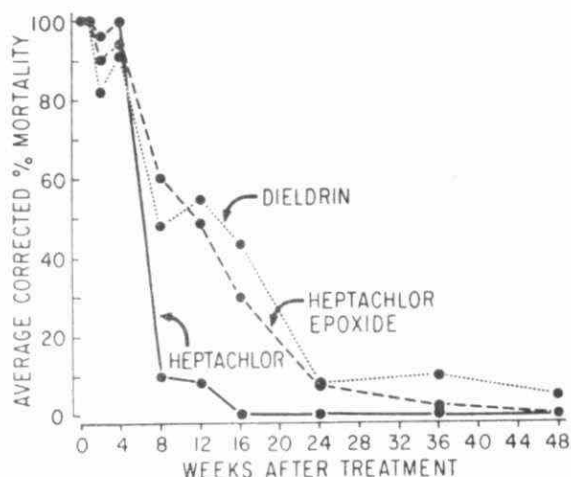


FIG. 3.—Persistence of biological activity of heptachlor epoxide as compared to heptachlor and dieldrin in Plainfield sand under controlled laboratory conditions.

epoxide. In muck soil as compared with the moist sandy loam, it was less effective by a factor of >20 . Other studies with the cyclodiene insecticides (Burkhardt and Fairchild 1967; Edwards 1966; Harris 1966, 1967, 1972a, b) indicated, that, in moist soils, organic content was the major factor influencing bioactivity. This was the case also with heptachlor epoxide (Table 3). Comparison of LD_{50} values obtained with moist sand and muck containing 0.5 and 70% organic matter showed that heptachlor epoxide was $\frac{1}{52}$ as effective in the latter. When plotted graphically the relationship between organic content and the LD_{50} was curvilinear, in contrast to other results obtained with dieldrin (Harris 1972a), and similar to results obtained with heptachlor (Harris 1966).

The preliminary screening tests indicated also that soil moisture influenced the bioactivity of heptachlor epoxide in mineral soil (Table 2). More detailed studies with the fine sandy loam at 6 moisture levels indicated that toxicity decreased with decreasing moisture (Fig. 1). The LD_{50} in soil containing 12% water was 0.25 ppm, in oven-dry soil it was 3.21 ppm, i.e. it was $\frac{1}{13}$ as effective in dry soil. The greatest effect of moisture on bioactivity occurred between 0 and 3% water; above this level moisture content was of minor importance.

Other studies (Harris 1971, 1972a, b) have indicated that soil temperature also influences the biological activity of organochlorine insecticides in soil. Toxicity of the cyclodiene insecticides aldrin, dieldrin, heptachlor, and chlordane is positively correlated with soil temperature, while that of DDT is

negatively correlated. Tests with heptachlor epoxide indicated that its behavior in relation to temperature is similar to that of the other cyclodiene insecticides. As a direct-contact insecticide LD_{50} values at the 4 temperatures used did not differ significantly (Table 4), indicating that within the range tested, temperature was not a factor influencing direct-contact toxicity. These results were similar to data previously obtained with aldrin and heptachlor but not with dieldrin which was twice as toxic at 33 as at 15°C when applied as a direct-contact insecticide. When applied as a residual-contact insecticide on a glass surface, heptachlor epoxide was twice as toxic at 33 as at 15°C. When compared with earlier data, its activity as a residual-contact insecticide was intermediate between aldrin and heptachlor on one hand and dieldrin on the other. It has been suggested (Harris 1971) that increasing toxicity with increasing temperature when an insecticide is applied as a residual-contact poison can probably be associated with increased mobility of the test insects with higher temperature and with increasing volatility. Since heptachlor epoxide appears to be intermediate in volatility between aldrin/heptachlor and dieldrin, these results can be explained on this basis. In soils the effect of temperature on bioactivity of heptachlor epoxide was more pronounced and was positively correlated with temperature (Fig. 2). The effect was least obvious in moist soils where toxicity increased 3.2 and 3.3 times in moist sandy loam and muck respectively, between 15 and 33°C. In the dry mineral soil, it was $21.5 \times$ more effective at 33 than at 15°C. When compared with earlier results, it is apparent that the effect of soil temperature on the biological activity of heptachlor is intermediate between aldrin/heptachlor on one hand and dieldrin on the other, with dieldrin being the insecticide most strongly influenced by soil temperature. Possible factors involved in the effects shown by temperature on insecticide activity in soil have been discussed elsewhere (Harris 1971).

Comparative tests on the persistence of biological activity of heptachlor, heptachlor epoxide, and dieldrin in Plainfield sand maintained at $27 \pm 1^\circ\text{C}$ showed that the heptachlor was less persistent than the epoxide (Fig. 3). With heptachlor, biological activity was nil after 16 weeks, while with the epoxide 48 weeks were required to obtain a 0 response. However, when compared with the data obtained with dieldrin it would appear that heptachlor epoxide is slightly less persistent in soil than dieldrin.

Field Studies.—These studies were set up with 2 objectives. First, the laboratory studies indicated that the major factor influencing the biological activity of heptachlor epoxide was soil type, specifically the organic content in moist soils. Second, previous studies with dieldrin (Harris and Sans 1972) had

Table 5.—Residues (ppm) of heptachlor epoxide in soil and in carrot roots and tops.

Soil type	% organic matter	Soil ^a					Carrots	
		Pretreatment	Initial	1 month	2 month	3 month	Roots	Tops
sand	0.9	0	1.81	2.00	1.89	1.88	0.563	0.123
1:1 sand:muck	16.6	0	2.10	2.26	2.12	2.08	.028	.012
muck	51.0	0	1.93	1.93	2.08	2.18	.009	.001

^a Sampling dates: May 29 (Pretreatment and Initial); July 3; Aug. 8; Sept. 3.

Table 6.—Vertical distribution of heptachlor epoxide (ppm) in treated soil^a and untreated subsoil.

Strata	Sand		1:1 sand:muck		Muck	
	I ^b	F ^c	I	F	I	F
0-5	1.78	1.32	2.27	2.21	2.47	1.88
5-10	1.89	1.79	2.13	1.93	2.14	2.02
10-15	1.79	1.57	2.44	2.22	2.40	2.34
15-20	<0.01	0.11	<0.01	<0.01	<0.01	<0.01
20-25	<.01	.04	<.01	<.01	<.01	<.01
25-30	<.01	<.01	<.01	<.01	<.01	<.01

^a Soil treated to a depth of 15 cm.

^b Initial sample.

^c 4 months after treatment.

indicated that both insecticidal activity in soils and uptake by plants were proportional to the organic content of the soil, and it seemed probable that heptachlor epoxide would respond similarly. Analysis of the pretreatment soil samples showed that no heptachlor epoxide was present in the soils prior to application of the EC formulation (Table 5). In addition, no residues were detected in the 3 control plots or on the carrots grown in those plots throughout the growing season. Following application of the EC, analysis of the initial soil samples taken immediately after treatment indicated residue levels ranging from 1.8 to 2.1 ppm. The residue levels did not decrease significantly throughout the summer.

Vertical movement of heptachlor epoxide through the soil profile was minimal (Table 6). The soil had been treated to a depth of 15 cm, and 4 months after treatment between 97 and 99% of the insecticide recovered was in the top 15-cm layer. The results also indicated that organic content of the soil was a limiting factor in that while limited leaching of the heptachlor epoxide occurred in the sand, virtually no movement occurred from the sand:muck and muck soils to the subsoil.

As expected, on the basis of the laboratory results, the insecticidal activity of heptachlor epoxide in the microplots was influenced also by soil organic content. In the sand containing 0.9% organic matter, the initial treatment of approximately 2 ppm resulted in 100% insect mortality at all sampling intervals when the samples were bioassayed in the laboratory. By contrast, in the sand:muck mix containing 16.6% organic matter, mortality (10%) occurred in only 1 of 4 samples taken during the growing season, while no mortality occurred in any of the muck assays.

Absorption of heptachlor epoxide residues from the soil by carrots was also dependent on soil type (Table 5). Although the 3 soils had ca. the same residue level, residues in carrots grown in the sand were ca. 20 times those grown in the sand:muck mix and >60 times those found in carrots grown in the muck soil containing 51% organic matter. Residues in the carrot tops were also inversely proportional to the organic content of the soil. Beall and Nash (1969) also reported that, in greenhouse experiments, organic content of the soil had the greatest negative influence on uptake of some organochlorine insecticides by seedlings.

The results obtained in the field study with heptachlor epoxide closely parallel data previously

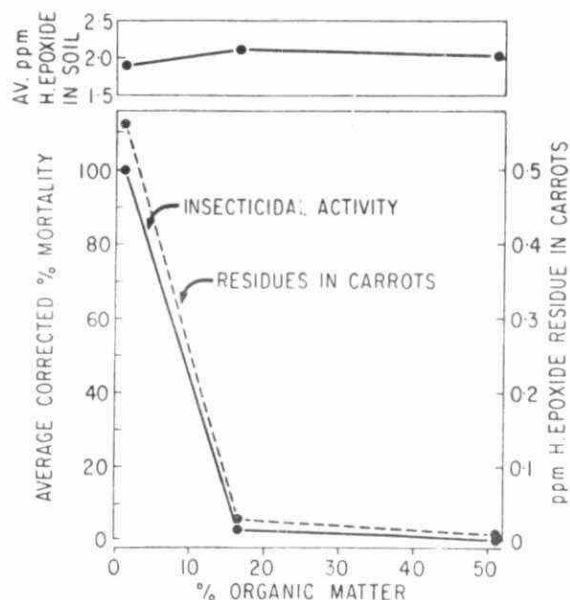


Fig. 4.—Influence of soil organic content on the insecticidal activity of heptachlor epoxide and its absorption by carrots.

obtained with dieldrin (Harris and Sans 1972) and can be best summarized graphically (Fig. 4). When heptachlor epoxide levels found in the 4 soil samples taken over the growing season were averaged for each soil type, the 3 average values were between 1.9 and 2.1 ppm. However, it was only in the sand containing 0.9% organic matter that the insecticide was insecticidally active and that carrots absorbed significant amounts of insecticide residue. In the soils containing higher levels of organic matter the insecticide was adsorbed and inactivated by the soil and thus was not available insecticidally (Fig. 4), for absorption by crops (Fig. 4), or for vertical movement down through the soil profile (Table 6). The significance of these results has been discussed elsewhere (Harris and Sans 1972).

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Factors Influencing the Biological Activity of Technical Chlordane and Some Related Components in Soil¹

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ABSTRACT

A study was conducted to determine the factors moderating the insecticidal activity of technical chlordane in soil. Direct-contact toxicity tests indicated that chlordane, while effective as a contact insecticide, was less toxic than aldrin or diazinon to 24- to 48-hr-old crickets, *Gryllus pennsylvanicus* (Burmeister), and adult picture-winged flies, *Chaetopsis debilis* (Loew). Both chlordane and aldrin were ineffective against 3rd-stage larvae of the darksided cutworm, *Euxoa messoria* (Harris). Soil type was the major factor influencing the biological activity of chlordane, and in moist soils toxicity was negatively correlated with organic content. Soil moisture was a factor of secondary importance. Bioactivity was positively correlated with temperature, but in moist mineral soil it was a factor of secondary importance. However, in dry mineral

soil temperature had a marked influence on toxicity. Studies on activity of some of the components and/or metabolites of technical chlordane indicated that, as contact poisons against crickets and flies, the order of toxicity was heptachlor>gamma-chlordane = alpha-chlordane = technical chlordane>nonachlor>chlordene>1-hydroxychlordene>hexachlorocyclopentadiene. In moist mineral soil the order was heptachlor>chlordane = gamma-chlordane>alpha-chlordane>nonachlor>chlordene>1-hydroxychlordene. Soil type and moisture influenced the activity of the insecticidally active components. Studies on the persistence of biological activity indicated that in Plainfield sand, nonachlor, alpha-, and gamma-chlordane were as persistent as dieldrin, while heptachlor, technical chlordane, and chlordene were similar in persistence to aldrin.

In recent years because of severe restrictions or outright bans on the use of the common organochlorine insecticides such as DDT, aldrin, and heptachlor, chlordane has been used more extensively than previously for soil-insect control. It has been pointed out in previous papers (Harris 1972, Harris and Sans 1972) that to achieve maximum soil-insect control with a minimum amount of insecticide, it will be necessary to obtain information on the specific behavior of an insecticide in relation to soil and climatic factors. A considerable amount of information has been published on the factors influencing the biological activity of several of the common organochlorine insecticides in soil (reviewed by Edwards 1966, Harris 1972). Much less is known about

the behavior of chlordane in soil. Most of the work done has been in relation to control of the Japanese beetle, *Popillia japonica* Newman, and the European chafer, *Amphimallon majalis* (Razoumowsky) (Fleming 1947, Gambrell et al. 1968). Residue studies related to these and other investigations have indicated that chlordane is intermediate in persistence between DDT and dieldrin on one hand and aldrin and heptachlor on the other (Bess and Hylin 1970, Lichtenstein and Polivka 1959, Onsager et al. 1970, Wiese and Basson 1966). Edwards (1966) estimated the average time for 95% disappearance of chlordane applied at 1-2 lbs AI/acre as 4 years, compared with aldrin at 3, dieldrin at 8, and DDT at 10 years. Nash and Woolson (1967), working under conditions which would have been such that the upper limit persistence of soil insecticides would be expected, determined the half-life of chlordane to be 8 years. They found also (Nash and Woolson 1968) that chlordane was one of the least mobile of the organochlorine insecticides and that very little vertical

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movement occurred below the cultivated layer. Less is known about the factors influencing the biological activity of chlordane in soil. However, Fleming and his colleagues (Fleming 1948, Fleming et al. 1962) established that soil temperature affected the "velocity of insecticidal action" of chlordane, and that organic content of the soil was a factor influencing bioactivity. Wiese (1964) found that chlordane became progressively less effective upon applications to soils with increasing clay content. It was the objective of the present study to further clarify the factors influencing the biological activity of technical chlordane in soil.

METHODS AND MATERIALS.—Procedures used have been described in detail in previous reports (Harris 1969, 1971, 1972) and thus are described only briefly here. Test insects included 24- to 48-hr-old crickets, *Gryllus pennsylvanicus* (Burmeister); adult picture-winged flies, *Chaetopsis debilis* (Loew); and 3rd-stage larvae of the darkspotted cutworm, *Euxoa messoria* (Harris). Unless otherwise specified the tests were done using crickets as the test insects.

Tests were conducted to determine the biological activity of reference-grade technical chlordane in soil and of some of its identifiable components and metabolites. GLC analysis indicated that the sample of reference-grade chlordane contained 21 components which responded on the electron capture detector. Of these, 3 were identified and quantitated: heptachlor 8.46%, alpha-chlordane 8.59%, and gamma-chlordane 10.53%. These 3 compounds therefore comprised 27.58% of the sample of reference-grade chlordane. These results were at variance with other data on the composition of technical chlordane. Spencer (1968) listed the composition of technical chlordane as consisting of "60-75% of the isomers of chlordane and 25-40% related compounds," while the Velsicol Corporation Standard for Technical Chlordane lists the approximate concentration of α -chlordane as $19 \pm 3\%$, γ -chlordane as $24 \pm 2\%$, and heptachlor as $10 \pm 3\%$. However, analysis of 2 other samples of chlordane available at this laboratory, a reference-grade sample obtained in 1957 and an EC purchased in 1966, gave results similar to those obtained with the 1968 reference grade sample used in these tests. In addition to these 3 components tests were conducted also on other known components of technical chlordane, including nonachlor, chlordene, and hexachlorocyclopentadiene, as well as on 1-hydroxy-chlordane which is a metabolite of heptachlor, and heptachlor epoxide in both soil and water (Carter and Springer 1970; Miles et al. 1969, 1971).

A Potter spray tower was used to assess the contact toxicity of technical chlordane; aldrin and diazinon were tested as standards for comparison. Tests were conducted against crickets, flies, and cutworms. Similar tests were conducted with the individual components and metabolites, using crickets as the test insects. Following treatment the insects were placed in observation containers and held under controlled conditions of temperature, humidity, and photoperiod.

Preliminary assessments of the activity of technical chlordane in soil were made by incorporating into the soil the insecticide, dissolved in distilled, chromatographed n-pentane, and evaporating the solvent. The treated soils were weighed into containers, moisture and food provided, test insects introduced, containers covered, and placed at the required environmental conditions. The preliminary tests were con-

ducted using a Beverly fine sandy loam (0 and 12% water (W)) and a muck (127% W). Aldrin and diazinon were used as standards to provide a basis for comparison. Similar tests were done with the individual components and metabolites using technical chlordane as a standard insecticide. Crickets were used as the test insects in all soil experiments.

The volatility of technical chlordane was assessed by measuring the fumigant effect to crickets placed on a gauze screen suspended 0.5 cm above the surface of a treated sandy loam. Aldrin and diazinon were used as standard insecticides. The insecticide concentrations applied to the soil were those determined to be the approximate LD₁₀₀ when the insects were placed directly on the soil.

In more detailed studies on the effects of soil type, moisture, and temperature on the biological activity of technical chlordane in soil, log dosage-probit lines were determined. Studies on the influence of soil type on biological activity were done using soils at the approximate field moisture capacity. Six soil types were used: a Plainfield sand, Beverly fine sandy loam, 2 samples of Brookston clay, and 2 samples of muck containing different levels of organic matter. Soil pH ranged from 6.7 to 7.9, while organic content ranged from 0.5 to 64.6%. Other characteristics of the soils have been described (Harris 1966). In addition, to provide a more suitable range of soil organic content, 4 mixtures of soil were prepared, combining the sand with the muck highest in organic content in ratios (by volume) of 2:1, 1:1, 1:2, and 1:3. The impact of soil moisture on the biological activity of technical chlordane was assessed using the fine sandy loam at 6 moisture levels: 0, 1, 3, 6, 9, and 12.3% water. The effect of temperature on biological activity also was assessed using the fine sandy loam which was preconditioned after treatment, for 1 hr, to obtain the required temperatures of 15, 21, 27, and 33°C. Tests were done at 2 moisture levels, 0 and 12.3% water.

Tests to determine the persistence of biological activity of technical chlordane and 5 of the components and/or metabolites were conducted using the procedures described elsewhere (Harris 1969). Dieldrin, aldrin, and diazinon were used as standards for comparison. The soil type was a Plainfield sand.

Table 1.—Direct-contact toxicity of chlordane, aldrin, and diazinon to 3 insect species.

Insecticide	Avg corrected % mortality at indicated % insecticide solution			
	0.001	0.01	0.1	1.0
<i>Crickets</i>				
Aldrin	0	68	100	100
Diazinon	0	16	100	100
Chlordane	0	0	85	100
<i>Flies</i>				
Diazinon	13	100	100	100
Aldrin	3	100	100	100
Chlordane	0	0	100	100
<i>Cutworms</i>				
Diazinon	0	0	5	90
Aldrin	0	0	0	0
Chlordane	0	0	0	0

Table 2.—Toxicity of chlordane, aldrin, and diazinon as soil insecticides (crickets).

Insecticide	Avg corrected % mortality at indicated ppm insecticide in soil						
	0.1	0.5	1	5	10	50	100
<i>Moist sandy loam</i>							
Aldrin	7	100	100	100	100	100	100
Diazinon	0	45	100	100	100	100	100
Chlordane	0	0	65	100	100	100	100
<i>Dry sandy loam</i>							
Aldrin	0	0	30	100	100	100	100
Chlordane	0	0	0	50	100	100	100
Diazinon	0	0	0	0	0	95	100
<i>Moist muck</i>							
Aldrin	0	0	0	100	100	100	100
Chlordane	0	0	0	0	100	100	100
Diazinon	0	0	0	0	0	100	100

The insecticide concentrations used were $2 \times$ the approximate LD_{50} ; i.e. nonachlor 13, α -chlordane, 3; γ -chlordane, 4; heptachlor, 0.6; technical chlordane, 3; chlordene, 16; dieldrin 1.2; aldrin, 0.6; and diazinon 1 ppm respectively. Soils were brought up to field capacity each week, and samples were removed at intervals up to 48 weeks. All tests were done under controlled laboratory conditions.

All tests were conducted using environmental chambers programmed to provide $27 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH, and 24-hr photoperiod, unless otherwise specified. Direct-contact toxicity tests were done using 4 insecticide concentrations: 0.001, 0.01, 0.1, and 1.0% solution. Preliminary screening tests comparing activity in soil were done using a wide range of 8 concentrations: 0.1, 0.5, 1, 5, 10, 50, 100, and 500 ppm (based on oven-dry weight of soil). Duplicate groups of 10 insects were used at each concentration. Each test was repeated a 2nd time, and the results were averaged. Corrections for natural mortality were made (Abbott 1925). In the more detailed studies, log dosage-probit lines were determined using 5–8 insecticide concentrations causing mortalities ranging from ca. 15 to 90%. Duplicate groups of 10 insects were used at each concentration. Each assay was run 3 times, and results were pooled prior to statistical analysis of the dosage-mortality data by computer. Controls using the solvent treatment only were run with each test.

RESULTS AND DISCUSSION.—Tests on the direct-contact toxicity of technical chlordane indicated that it was approximately $1/10$ as effective as aldrin and diazinon against both crickets and flies (Table 1). It was ineffective when applied to larvae of the dark-sided cutworm, as was aldrin, while diazinon caused mortality at the 1% concentration. Similar results were obtained in an earlier study (Harris and Svec 1970) which indicated that larvae of the dark-sided cutworm were tolerant to aldrin and chlordane when applied as soil and foliage applications. When incorporated into the moist sandy loam chlordane was $1/2$ as toxic as diazinon and $1/10$ as effective as aldrin (Table 2). At the concentrations at which they were active all 3 materials would be classed as effective soil insecticides. The tests on fumigant activity indicated that chlordane vaporized to a considerable extent. A concentration of 5 ppm in soil caused 100% mortality of the test insects suspended above the soil

surface in 18 hr. Aldrin and diazinon were also volatile, with 0.5 ppm aldrin and 1 ppm diazinon in soil causing 95 and 75% mortality respectively in the same period.

The preliminary screening tests (Table 2) indicated also that soil type was of major importance in moderating the biological activity of chlordane. In muck soil, chlordane was approximately $1/10$ as effective as in the sandy loam. Aldrin was inactivated to a similar extent, while diazinon was approximately $1/100$ as effective, indicating that it was more strongly adsorbed in the muck soil than either of the cyclodiene insecticides. The more detailed studies on the effect of soil type on the biological activity of chlordane indicated that, in moist soil, toxicity was negatively correlated with organic content of the soil (Table 3). In the moist Plainfield sand containing $1/2\%$ organic matter, the LD_{50} was 0.46 ppm. In the moist muck containing 64.6% organic matter, the LD_{50} was 18.82 ppm, a factor of 40.9. When plotted graphically the relationship between toxicity and organic content appeared to be linear. The 4 combinations of sand:muck fell fairly well in line with the results obtained with the 2 soils containing high levels of clay, thus indicating that in moist soils, clay content is of minor importance as a factor influencing the biological activity of nonpolar cyclodiene insecticides. Other studies, however, have demon-

Table 3.—Influence of soil type on the biological activity of technical chlordane (crickets).

Soil type and mixture	% W	pH	% organic matter			95% CL
			pH	LD_{50} (ppm)		
P. sand	8.7	7.6	0.5	0.46	0.13–0.48	
B. fine sandy loam	12.3	7.9	1.7	1.01	.96–1.06	
B. clay	23.0	7.3	5.0	1.17	1.12–1.22	
Sand:muck 2:1	25.0	7.3	5.8	1.37	1.30–1.45	
1:1	38.8	6.8	7.0	2.11	2.00–2.21	
1:2	53.8	7.0	10.4	3.47	3.24–3.66	
1:3	78.5	7.2	26.7	6.12	5.87–6.37	
B. clay	58.7	6.9	27.8	6.56	6.26–6.79	
Muck	96.0	6.7	42.3	11.61	10.91–12.19	
Muck	127.0	6.7	64.6	18.82	18.04–19.75	

Table 4.—Influence of soil moisture in a sandy loam on the biological activity of technical chlordane (crickets).

% W in soil	LD ₅₀ (ppm)	95% CL
0	5.33	4.93–5.71
1	2.09	1.98–2.20
3	1.54	1.46–1.62
6	1.25	1.15–1.33
9	1.03	0.97–1.08
12.3	1.01	.96–1.06

strated that when soil moisture levels are low, clay content can be a major factor in the inactivation of the cyclodiene insecticides in soil (Harris 1966, 1972). The results obtained in the soil-type studies indicated that the influence of soil type on the toxicity of chlordane closely paralleled results previously obtained with dieldrin (Harris 1972).

The preliminary screening tests (Table 2) indicated also that moisture was a factor influencing the toxicity of chlordane in soil, in that it was approximately $\frac{1}{3}$ as effective in the dry as compared to the moist soil. By contrast, aldrin was approximately $\frac{1}{10}$ as effective, and diazinon $\frac{1}{100}$ as effective, thus indicating that moisture was of less importance to the activity of chlordane in soils than the other 2 materials. More detailed studies using the sandy loam indicated that chlordane was less effective in the dry soil as compared with the moist soil by a factor of 5.3 (Table 4). The greatest portion of this effect occurred between 0 and 3% water; above this level, differences in the LD₅₀ values were of little consequence. Comparison of these results with earlier studies on the same soil type and under the same conditions (Harris 1966, 1971, 1972; Harris and Sans 1972) indicated that with the common organochlorine soil insecticides, soil moisture had the greatest effect on dieldrin > DDT > heptachlor epoxide = heptachlor > aldrin > chlordane.

Previous studies have shown that aldrin, heptachlor, heptachlor epoxide, and dieldrin were less effective in dry mineral soil at 15 as compared with 33°C by factors of 12.9, 14.7, 21.5, and 26.2 (Harris 1971, 1972), whereas the present study showed chlordane to be less effective in dry mineral soil by a factor of 133 (Table 5). Thus in dry mineral soil the temperature effect on the biological activity of chlordane is much more pronounced than with the other cyclodiene insecticides. In moist sandy soil chlordane was less toxic at the lower temperature by a factor of 3.9, and the previous studies with the other cyclodiene insecticides have shown that toxicity is also positively correlated with temperature but to a lesser degree than in dry soils. The relation to activity showed a gradient of: dieldrin = chlordane = heptachlor epoxide > aldrin > heptachlor.

Since the biological activity of technical chlordane in soil represents the joint action of its various insecticidal components, it was of interest to determine the activity of these compounds both as direct contact and soil insecticides. Results of the direct-contact-toxicity tests (Table 6) indicated that heptachlor was ca. 10 × more toxic than chlordane to both crickets and flies. Gamma- and alpha-chlordane were slightly more effective against both species of

Table 5.—Influence of soil temperature on the biological activity of technical chlordane in a sandy loam (crickets).

% W in soil	Temp °C	LD ₅₀ (ppm)	95% CL
12.3	15	3.31	3.19– 3.45
	21	1.75	1.69– 1.80
	27	1.01	0.96– 1.06
	33	0.84	.82– 0.87
0	15	278.0	264.6–292.7
	21	63.52	60.00– 67.05
	27	5.33	4.93– 5.71
	33	2.08	1.96– 2.19

insects than technical chlordane, while nonachlor was slightly less effective. Hexachlorocyclopentadiene was nontoxic at the concentrations tested. The results obtained with chlordane and 1-hydroxychlordane were of interest, since it has been shown in *in vitro* studies that soil microorganisms can degrade heptachlor and heptachlor epoxide to either chlordane or 1-hydroxychlordane (Miles et al. 1969, 1971). These present results indicated that both chlordane and 1-hydroxychlordane were ca. $\frac{1}{10}$ as toxic insecticidally as technical chlordane and $\frac{1}{100}$ as toxic as heptachlor.

In moist sandy loam heptachlor was ca. 10× as effective, gamma-chlordane equitoxic, and alpha-chlordane slightly less toxic than technical chlordane (Table 7). Nonachlor and chlordane were ca. $\frac{1}{10}$ as toxic, while 1-hydroxychlordane was nontoxic even when applied to the soil at the rate of 500 ppm. As would be expected, on the basis of the data obtained with technical chlordane, both soil moisture and type influenced the toxicity of the various components (Table 7). Comparison of the results obtained

Table 6.—Direct contact toxicity of technical chlordane, and some of its individual components to 24–48-hr crickets and flies.

Compound	Avg corrected % mortality at indicated % insecticide solution			
	0.001	0.01	0.1	1.0
<i>Crickets</i>				
Tech. chlordane	0	0	85	100
Heptachlor	0	22	100	100
Gamma-chlordane	0	0	100	100
Alpha-chlordane	0	0	95	100
Nonachlor	0	0	56	100
Chlordane	0	0	0	100
1-Hydroxychlordane	0	0	0	87
Hexachlorocyclopentadiene	0	0	0	3
<i>Flies</i>				
Tech. chlordane	0	0	98	100
Heptachlor	0	78	100	100
Gamma-chlordane	0	0	100	100
Alpha-chlordane	0	0	100	100
Nonachlor	0	0	63	100
Chlordane	0	0	0	100
1-Hydroxychlordane	0	0	0	0
Hexachlorocyclopentadiene	0	0	0	0

Table 7.—Activity of technical chlordane and some of its individual components as soil insecticides (crickets).

Compound	Avg corrected % mortality at indicated ppm insecticide in soil							
	0.1	0.5	1	5	10	50	100	500
<i>Moist sandy loam</i>								
Tech. chlordane	0	0	50	100	100	100	100	
Heptachlor	20	100	100	100	100	100	100	
Gamma-chlordane	0	0	50	100	100	100	100	
Alpha-chlordane	0	0	5	100	100	100	100	
Nonachlor	0	0	0	75	95	100	100	
Chlordene	0	0	0	20	90	100	100	
1-Hydroxychlordene	0	0	0	0	0	0	0	0
<i>Dry sandy loam</i>								
Tech. chlordane	0	0	0	65	100	100	100	
Heptachlor	0	0	20	100	100	100	100	
Gamma-chlordane	0	0	0	0	0	100	100	
Alpha-chlordane	0	0	0	0	0	100	100	
Chlordene	0	0	0	0	0	65	100	
Nonachlor	0	0	0	0	0	10	80	
1-Hydroxychlordene	0	0	0	0	0	0	0	0
<i>Moist muck</i>								
Tech. chlordane	0	0	0	0	30	100	100	
Heptachlor	0	0	0	100	100	100	100	
Gamma-chlordane	0	0	0	0	45	100	100	
Alpha-chlordane	0	0	0	0	20	100	100	
Nonachlor	0	0	0	0	0	95	100	
Chlordene	0	0	0	0	0	20	100	
1-Hydroxychlordene	0	0	0	0	0	0	0	0

on moist and dry sandy loam indicated that technical chlordane was less effective in the dry soil by a factor of 5, heptachlor, chlordene, and nonachlor by factors of 10, and gamma- and alpha-chlordane by factors of approximately 50. 1-Hydroxychlordene was ineffective in dry mineral soil at 500 ppm. The soil type tests indicated that technical chlordane was less effective in muck soil as compared with sandy loam by a factor of 10. This was the case also with the other materials, with the exception of the 1-hydroxychlordene which was ineffective even at 500 ppm.

The tests on persistence of biological activity were done using Plainfield sand. Dieldrin, aldrin, and diazinon were used as standard insecticides, since they may be classed as highly, moderately, and slightly persistent, respectively. The results of the tests on technical chlordane and its components indicated that nonachlor, alpha-chlordane, and gamma-chlordane were similar in persistence of biological activity to dieldrin, i.e. highly residual in soil (Fig. 1). When compared with earlier data (Harris and Sans 1972), alpha- and gamma-chlordane appeared to be more persistent in soil than heptachlor epoxide. Heptachlor, technical chlordane, and chlordene were similar to aldrin in that biological activity disappeared within 24 weeks following treatment, i.e. they were moderately residual compounds. It should be noted that by using a soil low in organic matter and clay minerals the data obtained represent minimum persistence values. In heavy mineral soils, or in soils containing higher levels of organic matter, the persistence of the chemical per se would be proportionately longer. At the same time, it is unlikely that such residues would be biologically active.

It has been noted here that GLC analysis of the sample of reference-grade technical chlordane indicated that the 3 major insecticidal components, hep-

tachlor, α -chlordane, and γ -chlordane, comprised 27.58% of the sample and were present in ca. equal amounts. As a contact poison (Table 6), heptachlor was ca. 10 \times as toxic to crickets as either technical, α , or γ -chlordane. Considering that the compounds were present in approximately the same amounts, it would appear that the activity of the technical chlordane as a contact poison was due primarily to the presence of 8.59% heptachlor in the sample. This conclusion is further substantiated by the data obtained in soil (Table 7). Heptachlor was again ca. 10 \times as toxic as either technical α , or γ -chlordane in moist sandy loam, and the 3 last-mentioned materials were all toxic at ca. 1 ppm. Considering the composition of technical chlordane, soil treated at 1 ppm would contain 0.085 ppm heptachlor, 0.085 ppm α -chlordane, and 0.105 ppm γ -chlordane. From the data given in Table 7, it is apparent that while 0.085 ppm heptachlor would be close to the concentration of 0.1 ppm which caused insect mortality in soil, the α - and γ -chlordane concentrations would be ca. $\frac{1}{10}$ the level required to kill. The data on persistence of biological activity (Fig. 1) also support the conclusion that the insecticidal activity of technical chlordane is due primarily to the presence of heptachlor. Both heptachlor and technical chlordane were classed as moderately persistent compounds, while α - and γ -chlordane were highly persistent. If the latter 2 compounds were contributing significantly to the insecticidal activity of technical chlordane, the persistence curves would have been intermediate between heptachlor on one hand and α - and γ -chlordane on the other. While the toxicity of technical chlordane will be dependent on the combined actions of these 3 compounds, it is apparent that the 8.5% heptachlor content is largely responsible for its insecticidal action.

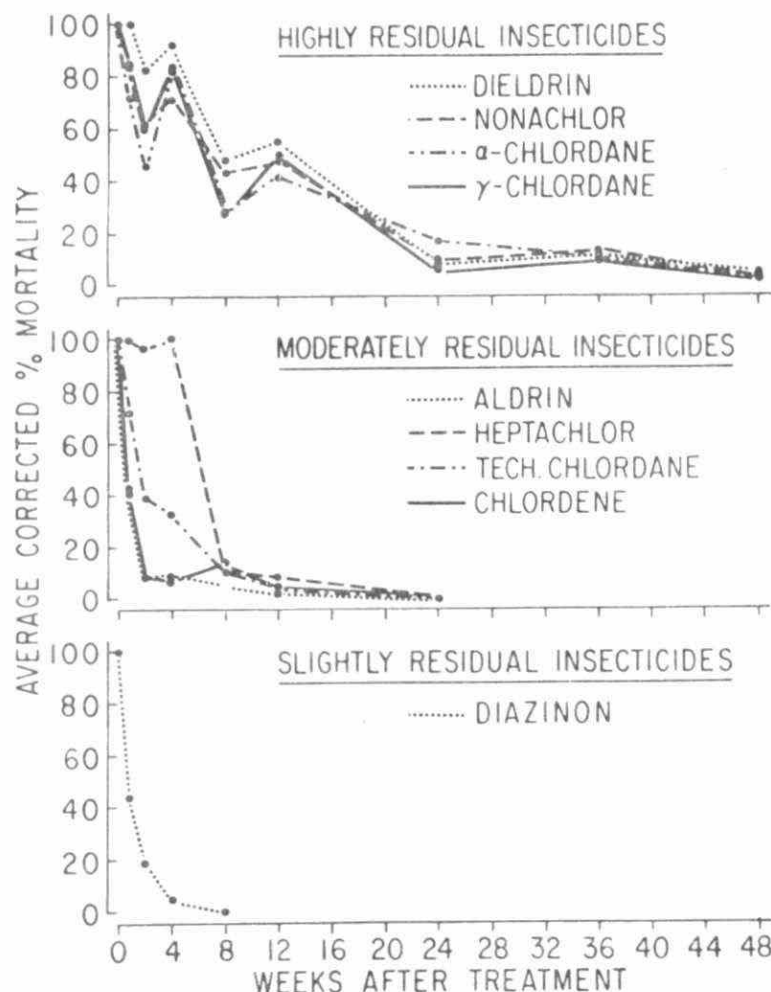


FIG. 1.—Persistence of biological activity under controlled laboratory conditions of technical chlordane and some of its individual components in Plainfield sand, as compared with aldrin, dieldrin, and diazinon (crickets).

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Insecticide Residues in Soils on 16 Farms in Southwestern Ontario—1964, 1966, and 1969¹

C. R. Harris and W. W. Sans

ABSTRACT

A study was conducted on 16 farms in southwestern Ontario during 1964, 1966, and 1969 to determine the extent to which residues of insecticides were accumulating in agricultural soils as a result of current insect control practices. Residues of organochlorine insecticides were determined by gas-liquid chromatography (GLC), while those of the organophosphorus insecticides were determined qualitatively by non-specific enzymatic analysis and quantitatively by GLC where possible. Residues of the organochlorine insecticides were present in soils on all 16 farms in 1964, 1966, and 1969. DDT/DDE/DDD occurred on all farms, aldrin/dieldrin on 14 of 16, and heptachlor/heptachlor epoxide/ γ -chlordane on 6 farms. Other organochlorine insecticides detected included dicofol in orchards, endrin, trace amounts of lindane, and endosulfan. Highest average residue levels of the organochlorine insecticides were present in orchard > vegetable > tobacco > field crop soils. The use pattern indicated that the organochlorine insecticides were used almost exclusively between 1961 and 1964, but that the organophosphorus insecticides received increased use from 1965-1969. Residues of the organochlorine insecticides in soil appeared to be consistent with the use pattern in that they were highest in 1966 and declined by 1969 to levels similar to those found in 1964. Preliminary data indicated that the trend to extensive use of the organophosphorus insecticides is resulting in the presence of residues of some of these materials in vegetable soils.

Introduction

For over 2 decades, the organochlorine insecticides have been used extensively to control insects attacking agricultural crops. While these materials have proved to be

highly effective against both soil and foliar insects, some are persistent, and residues of these compounds or their metabolites are known to accumulate in soils (1). In Canada, DDT has been used extensively since it became available, and since soil insects have been a particularly serious problem, the cyclodiene insecticides were also used extensively between 1954 and 1960 but at a decreasing rate since that time.

Some areas of the country, e.g., the Prairie Provinces, have received large scale applications of pesticides, but at irregular intervals and at relatively low rates of application. By contrast, in southwestern Ontario, an area of intensive agriculture with a broad spectrum of soil types and high value cash crops, pesticides are applied regularly in concentrated areas at relatively high rates. A study in 1964 (7) on 31 farms in southwestern Ontario indicated that residues of the organochlorine insecticides were present in nearly all soils, with the most common being: technical DDT and its metabolites DDE and DDD > aldrin/dieldrin > heptachlor/heptachlor epoxide/ γ -chlordane > endrin. The highest residues were found in orchard > vegetable > tobacco > other field crop soils. The study was continued through 1969 on 16 of the original 31 farms to determine to what extent insecticide residues were accumulating in agricultural soils as a result of the insect control practices during that time. This report summarizes the data obtained on the persistence of organochlorine insecticides in soils on these 16 farms for 1964, 1966, and 1969 and also provides some preliminary data on the occurrence in soils of some organophosphorus insecticides used as replacements for DDT and the cyclodiene insecticides.

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Methods and Materials

The 16 farms selected for the study were in areas of very intensive agriculture requiring extensive use of insecticides. Care was taken to select cooperators who would adhere closely to the registered or recommended uses of insecticides. Crops included fruit, tobacco, and a wide range of vegetable and field crops. Soil type varied considerably, with orchards on sandy to clay loam, tobacco on sand to sandy loam, vegetables on sandy loam or muck, and field crops on sandy loam or heavier mineral soils (Table 1). Each cooperator was interviewed, and as much as possible, a 10-year history of cropping practices and insecticide treatments was obtained (Table 1). While such data serve as a useful guideline, experience has shown that information obtained in this manner is often erroneous, particularly with regard to insecticide use. After initiation of the study in 1964, the cooperators were asked to keep more accurate records of insecticide use, and thus the 1965-69 data are more representative of use patterns than the pre-1964 data.

SAMPLING PROCEDURES

Soil samples were collected in 1964, 1966, and 1969. Each sampling site, comprising an area of approximately 5 acres within a field, was mapped out by measurement from permanent landmarks in order to assure returning to the exact site in ensuing years. Five subareas were sampled within the 5-acre site. The subareas, which were 4 feet square, were placed diagonally to the perimeter of the field. Twenty-five 6- by 1-inch cores were taken from each subarea; the cores from all subareas were pooled in order to obtain a representative sample of the field. The pooled sample (approximately 10 lb of soil) was sealed in a plastic bag and refrigerated at 2°C. In orchards, samples were taken both between and under the trees and analyzed separately, but for the purposes of this study the results have been averaged. Samples were taken in October and November of each year. During the course of the study, two farms ceased to be used for agriculture (Table 1). Farm No. 9 became part of a housing development in 1967 and therefore was not sampled in 1969. Farm No. 15 was converted to a municipal park in 1968, however, samples were collected from the park in 1969.

ANALYTICAL PROCEDURES

The procedures for extraction, fractionation, and analysis were designed primarily for the organochlorine insecticides. Although data are also presented on organophosphorus insecticide residues in soil, it should be noted that subsequent experience has indicated that these procedures are not adequate for some organophosphorus insecticides therefore, the data on these compounds should not be considered complete.

Insecticide residues were extracted from the soil within a few days of sampling. Two hundred milliliters of distilled acetone was added to 200 g of moist soil (water content adjusted to approximately 50% field moisture capacity) in 16-oz screw cap bottles. The bottles were swirled to obtain good distribution of the acetone:soil mixture, and 200 ml of distilled petroleum ether or hexane was added. The bottles were capped and tumbled on an end-over-end tumbler for 1 hour at approximately 29 rpm, then the supernatant liquid was transferred to 2-liter separatory funnels and the acetone removed by washing three times with distilled water. (Subsequent experience has shown that more polar organophosphorus insecticides such as fensulfothion (Dasanit) remain with the acetone: water mixture and are therefore discarded). The hexane phase was passed through anhydrous sodium sulfate and collected in 8-oz screw cap bottles. The extracts were stored at -10°C until analyzed. Recovery values, obtained by adding known amounts of insecticide standards to residue-free sandy loam and muck soils followed by evaporation of the solvent prior to extraction, indicated >90% recovery for all the organochlorine insecticides and for some organophosphorus insecticides such as diazinon, Dursban, dichlofenthion (Nemacide), and parathion. Recovery data using this extraction procedure were not obtained for the less common organophosphorus and carbamate insecticides or the metabolites. Since the soil types used in the recovery studies were not representative of the wide range of soils sampled in this study, nor of weathered samples, no corrections were made for percent recovery.

Injection of crude extracts from soil into a gas chromatograph can result in poor definition of peaks when several insecticides are present, misinterpretation of other soil components or contaminants as insecticides, and rapid degeneration of GLC column and detector efficiency. Consequently, all samples were cleaned up and fractionated on Florisil columns. The technique has been described in detail (12) and will be outlined only briefly here. The column was eluted with four solvents as follows: 200 ml of petroleum ether (first fraction); 200 ml of 5:1 benzene:petroleum ether (second fraction); 200 ml of chloroform (third fraction); and 150 ml of acetone (fourth fraction). The first fraction contained residues of heptachlor, aldrin, *o,p'*-DDT, *p,p'*-DDT, and DDE. The second fraction contained lindane, heptachlor epoxide, γ -chlordane, dieldrin, endrin, DDD, methoxychlor, dicofol, and endosulfan. The majority of the organophosphorus insecticides or their metabolites appeared in the third and fourth fractions, but some chlorinated organophosphorus insecticides eluted in the second fraction. The eluates were concentrated to approximately 2 ml in a rotary evaporator, the residue taken up in hexane, and transferred to a 10-ml volumetric flask.

TABLE 1.—Crop history and insecticide usage for the 16 farms studied, 1960–69

FARM No.	GENERAL CLASSIFICATION (CROPS)	SOIL TYPE		CROP HISTORY AND INSECTICIDE USAGE									
				1960	1961	1962	1963	1964	1965	1966	1967	1968	1969
1	Field	Clay loam	Crops	alfalfa	alfalfa	corn	corn	corn	corn	corn	corn	wheat	corn
			Insecticides	—	—	aldrin	aldrin	aldrin	aldrin	aldrin	—	—	—
2	Field	Sandy loam	Crops	no data	corn	oats	alfalfa	sugar beets	corn	oats, alfalfa	alfalfa	alfalfa	soybeans
			Insecticides	no data	H (seed tr.)	—	—	—	—	—	—	—	—
3	Field	Loam	Crops	fallow	turnips	corn	turnips	wheat	oats	oats, alfalfa	potatoes	corn	corn
			Insecticides	—	aldrin	—	aldrin	—	—	—	DDT	—	—
4	Field	Clay	Crops	turnips	clover	fallow	oats	turnips	wheat	wheat	corn, cabbage	rye, cabbage	turnips, corn
			Insecticides	aldrin	—	—	—	aldrin	aldrin	aldrin	mev	mev	fen, C
5	Tobacco	Sandy loam	Crops	rye, tobacco	potatoes	rye	tobacco	rye	tobacco	potatoes, wheat	potatoes	tobacco	potatoes
			Insecticides	no data	no data	no data	no data	no data	aldrin	endrin, DDT	C	C	C, endrin
6	Tobacco	Sand	Crops	rye	tobacco	rye	tobacco	wheat	tobacco	wheat	tobacco	wheat	tobacco
			Insecticides	—	DDT	—	DDT	—	DDT	—	DDT	—	DDT
7	Tobacco	Sand	Crops	tobacco	rye	tobacco	wheat	tobacco	wheat	tobacco	rye	tobacco	rye
			Insecticides	aldrin, DDT	—	DDT	—	DDT	—	DDT	—	DDT	—
8	Tobacco	Sandy loam	Crops	tobacco	rye	tobacco	rye	tobacco	rye, tobacco	rye, tobacco	rye, tobacco	rye, tobacco	rye, tobacco
			Insecticides	aldrin	—	H, DDT	—	DDT, endrin	DDT, C	C	DDT, C	DDT, C	DDT, C, Dur
9	Vegetables	Sandy loam	Crops	onions	onions	onions	lettuce	lettuce	tomatoes	lettuce	no data	no data	no data
			Insecticides	aldrin	aldrin	aldrin	DDT	DDT	—	—	no data	no data	no data
10	Vegetables	Muck	Crops	onions	onions	onions	onions	onions	onions	onions	onions	onions	onions
			Insecticides	aldrin, H	aldrin, H	D, H	D, H	D, H	Dz, dichlo	onions, dichlo, aldrin	DDT, dichlo	DDT	DDT
11	Vegetables	Muck	Crops	radishes	radishes	radishes	radishes	radishes	sorghum	cucumbers, corn	corn	corn	radishes
			Insecticides	endrin, DDT	endrin, DDT	endrin, DDT	endrin, DDT	endrin, DDT	—	endo, aldrin	—	—	DDT, P
12	Vegetables	Muck	Crops	onions	celery	celery	onions	onions	celery	onions	celery, onions	celery, onions	celery, onions
			Insecticides	DDT	DDT	DDT	DDT	DDT	mev, DDT	Dz	DDT, endo, mev	DDT, endo, mev	DDT, endo, mev
13	Vegetables	Sandy loam	Crops	radishes	radishes	radishes	radishes	radishes	onions	beets	onions, radishes	onions, radishes	onions, radishes
			Insecticides	aldrin	aldrin	aldrin	aldrin	aldrin	ethion	—	ethion, P, DDT	ethion, P	ethion, P
14	Vegetables	Muck	Crops	carrots	carrots	lettuce	onions	carrots	carrots	onions	carrots	onions	lettuce, radishes
			Insecticides	DDT	DDT	DDT	DDT	DDT	DDT, Dz	DDT, Dz, dichlo	DDT, Dz, dichlo	DDT, Dz, dichlo	C, mal
15	Fruit	Sandy loam	Crops	apples	apples	apples	apples	apples	apples	apples	apples	none	none
			Insecticides	DDT	DDT	DDT	DDT	DDT	no data	no data	—	—	—
16	Fruit	Sandy loam	Crops	apples	apples	apples	apples	apples	apples	apples	apples	apples	apples
			Insecticides	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data

NOTE: C = carbaryl
D = dieldrin
Dz = diazinon
dichlo = dichlofenthion
Dur = Dursban
endo = endosulfan

fen = fen sulfathion
H = heptachlor
mal = malathion
mev = mevinphos
P = parathion

Organochlorine insecticide residues were determined using GLC. Studies in 1964 were carried out using a Wilkins Aerograph Model 600C Hy-Fi gas chromatograph and an additional oven, Model 550, equipped with electron capture detectors; subsequent studies used a Varian Aerograph Model 205B dual column GC and a Model 1200 single column GC equipped with electron capture detectors. Operating parameters have been described in detail elsewhere (12). In 1964, 2-column packings were used: DC-11 (5% on Chromosorb W) for identification and quantitation and QF-1 (5% on Aeropak 30) for additional verification. In the following years a DC-200 (5% on Aeropak 30) column was used in place of the DC-11 column. In cases where identification was still in doubt, chemical conversion techniques prior to GLC were also utilized (12). The samples were analyzed for: heptachlor, heptachlor epoxide, γ -chlor-dane, aldrin, dieldrin, endrin, *o,p'*-DDT, *p,p'*-DDT, DDE, DDD, dicofol, methoxychlor, endosulfan, and lindane. Results obtained for *o,p'*- and *p,p'*-DDT are reported as technical DDT. Sensitivity of the techniques was 0.01 ppm in 1964 and 0.001 ppm in 1966 and 1969. Results are reported in parts per million based on the oven-dry weight of the soil.

Organophosphorus insecticide residues which had been extracted were detected qualitatively by enzymatic analysis and quantitatively, when possible, by GLC. Enzymatic analyses were done on fractions 3 and 4 of the Florisil eluate which were known to contain the majority of the organophosphorus insecticides or their metabolites. The technique used was a modification of the methods of Giang and Hall (2) and Hensel *et al.* (8). Two solutions were prepared: (a) a buffered pseudo-acetylcholinesterase solution utilizing outdated human blood plasma and (b) a 0.132 M acetylcholine bromide solution. One milliliter aliquots of fractions 3 and 4 of the eluates from the Florisil columns were placed in 5-ml beakers. Control samples and control samples plus known concentrations of an enzyme-inhibiting insecticide (diazinon) were also prepared. All samples were replicated. The samples were evaporated just to dryness and 2 ml of Solution (a) added to the beakers at precisely timed 2-minute intervals. The beakers were incubated in a water bath at $37 \pm 0.5^\circ\text{C}$ for 70 minutes, stirring at 15-minute intervals. The samples designated for the initial pH readings were read at that time. One milliliter of Solution (b) was added to the remaining beakers which were then incubated for another 2 hours. Final pH readings were taken and percent inhibition was calculated as follows:

$$\text{Percent inhibition} = \frac{\Delta \text{pH of control sample} - \Delta \text{pH of treated sample}}{\Delta \text{pH of control sample}} \times 100$$

$$(\Delta \text{pH} = \text{pH}_{\text{initial}} - \text{pH}_{\text{final}})$$

When possible, the presence of organophosphorus insecticide residues in the soil was determined quantitatively by GLC utilizing the equipment and techniques outlined above. Verification was made using a Wilkins Hy-Fi Model 600C GC equipped with an alkali flame ionization detector using cesium bromide or rubidium sulfate salts.

Results and Discussion

Information obtained from those cooperators who could provide data on insecticide use indicated that, in nearly all cases, insecticides had been used extensively between 1960 and 1969 (Table 1). An exception was on Farm No. 2 where the only reported insecticide use had been a heptachlor seed treatment in 1961. Between 1961 and 1964, the organochlorine insecticides were used almost exclusively; from 1965-69, more emphasis was placed on use of organophosphorus insecticides. The greatest pesticide usage was on fruit, vegetable, and tobacco crops, with few insecticide requirements for field crops. The cyclodiene insecticides were used primarily for soil insect control—including the seed-corn maggot, *Hylemya platura* (Meigen); the cabbage maggot, *H. brassicae* (Bouche); the onion maggot, *H. antiqua* (Meigen); the black cutworm, *Agrotis ipsilon* (Hufnagel); the variegated cutworm, *Peridroma saucia* (Hubner); the northern corn rootworm, *Diabrotica longicornis* (Say); and several species of wireworms. Aldrin was the insecticide most used, with heptachlor and endrin used to a lesser extent. DDT was used extensively for control of the dark-sided cutworm, *Euxoa messoria* (Harris) in tobacco, as well as the other cutworm species listed above. It was also used extensively for controlling a wide range of foliar insects on tobacco, vegetables, and fruit. In some cases, e.g., on tobacco, a single soil application was applied annually. In other cases, e.g., vegetables and orchards, numerous applications were made in a single year. Between 1958 and 1964, the seed-corn, onion, and cabbage maggots all developed resistance to the cyclodiene insecticides (6,9,10) as did the dark-sided cutworm attacking tobacco (6). As a result, use of the cyclodiene insecticides decreased significantly by 1965. The main use between 1965 and 1969 was for control of the northern corn rootworm. Aldrin, dieldrin, and heptachlor were banned from agricultural use in the Province of Ontario in May 1969. DDT received extensive use up to 1969, particularly for cutworm control in tobacco and on vegetable crops; however, other recommended uses decreased markedly between 1965 and 1969, and it was banned, with two exceptions, from use in Ontario in January 1970. The data in Table 1 also indicate that insecticides receiving increased use in place of the organochlorine insecticides include mevinphos, fensulfothion, carbaryl, Dursban, diazinon, dichlofen-thion, endosulfan, parathion, malathion, and ethion.

The results (Table 2) show that DDT plus metabolites were present in the soil on all 16 farms, with the smallest amounts on Farms 1 and 2 which had been devoted solely to field crops. The highest residues were found in the two orchards on Farms 15 and 16. On Farm 15, residues of technical DDT reached a peak of 97 ppm in

1966. Aldrin and dieldrin were found in 14 of 16 soils in amounts ranging from a trace (<0.001 ppm) to as high as 2.3 ppm of aldrin and 2.5 ppm dieldrin on Farm 11 in 1969. Heptachlor/heptachlor epoxide/ γ -chlordane were present in significant amounts on six of the farms. Endrin was found on only two farms in 1964; by 1969,

TABLE 2.—Residues of organochlorine insecticides found in agricultural soils on 16 farms in southwestern Ontario in 1964, 1966, and 1969

SAMPLE No.	YEAR	RESIDUES IN SOIL (PPM) ¹									
		HEPTACHLOR	HEPTACHLOR EPOXIDE	γ -CHLORDANE	ALDRIN	DIELDRIN	ENDRIN	DDT ²	DDE	DDD	DICOPOL
1	1964	—	—	—	0.51	0.40	—	—	—	—	—
	1966	T	T	T	0.22	0.23	0.01	0.02	T	T	—
	1969	T	T	T	0.29	0.31	0.01	0.02	T	T	—
2	1964	—	—	—	T	T	—	—	—	—	—
	1966	—	—	—	0.03	0.29	—	0.03	0.01	T	—
	1969	—	—	—	T	0.11	0.10	0.03	0.21	T	—
3	1964	—	—	—	0.51	0.85	—	0.32	—	—	—
	1966	—	—	—	0.29	1.29	—	0.38	0.15	T	—
	1969	—	—	—	0.10	1.16	—	0.93	0.15	T	—
4	1964	—	—	—	0.17	1.05	—	2.64	T	—	—
	1966	—	—	—	0.09	1.10	—	0.40	0.12	T	—
	1969	—	—	—	0.04	1.13	—	0.30	0.06	0.01	—
5	1964	—	—	—	0.23	0.57	0.11	0.95	—	—	—
	1966	—	—	—	0.14	0.64	0.10	0.95	0.09	T	—
	1969	—	—	—	0.01	0.30	0.06	0.26	0.09	0.02	—
6	1964	T	T	T	T	0.31	—	3.80	0.25	—	—
	1966	0.03	0.16	0.06	0.02	0.33	0.10	4.97	0.48	0.16	—
	1969	T	0.04	0.03	0.02	0.17	0.07	4.57	0.88	0.05	—
7	1964	T	T	0.10	T	0.32	—	4.58	0.05	0.06	—
	1966	0.40	0.10	0.13	0.01	0.51	0.03	4.55	1.00	0.03	—
	1969	0.01	0.08	0.03	0.01	0.48	0.03	1.88	0.79	0.10	—
8	1964	T	0.15	0.17	T	0.21	—	2.11	0.32	0.10	—
	1966	0.02	0.14	0.13	T	0.17	0.05	4.63	1.01	0.06	—
	1969	T	0.07	0.07	T	0.10	0.04	4.03	0.77	0.08	—
9	1964	T	T	0.19	T	1.26	—	3.44	0.15	T	—
	1966	0.02	0.14	0.13	0.48	1.52	—	2.34	0.33	0.06	—
	1969	—	—	—	—	—	—	—	—	—	—
10	1964	0.24	T	0.63	T	1.11	—	4.60	0.42	—	—
	1966	0.23	0.19	0.55	0.08	3.33	0.38	10.89	1.14	0.17	—
	1969	0.07	0.05	0.22	0.07	1.19	0.08	5.75	0.25	0.14	—
11	1964	—	—	—	2.13	1.58	3.76	13.80	0.75	0.38	—
	1966	—	—	—	1.23	2.52	6.55	15.23	0.74	0.95	—
	1969	—	—	—	2.33	2.54	3.54	7.52	0.81	0.79	—
12	1964	—	—	—	T	—	—	22.64	1.09	0.27	—
	1966	—	—	—	0.73	0.19	—	34.69	1.41	0.21	—
	1969	—	—	—	0.19	0.21	—	38.18	2.29	0.29	—
13	1964	T	T	—	0.79	0.67	—	0.59	0.11	—	—
	1966	0.04	0.24	0.18	0.11	0.54	—	0.22	0.14	T	—
	1969	0.04	0.16	0.13	0.08	0.67	—	0.21	0.05	—	—
14	1964	—	—	—	T	0.78	—	45.36	1.65	0.58	—
	1966	—	—	—	0.17	1.29	—	96.20	4.00	1.22	—
	1969	—	—	—	0.04	0.87	1.06	43.42	3.07	1.30	—
15	1964	—	—	—	—	—	—	93.35	8.40	2.70	4.93
	1966	—	—	—	—	—	—	97.07	9.94	1.78	2.97
	1969	—	—	—	—	—	—	75.94	8.39	2.04	4.93
16	1964	—	—	—	—	—	—	66.70	12.65	3.30	3.10
	1966	—	—	—	—	—	—	62.50	7.84	1.48	2.44
	1969	—	—	—	—	—	—	23.11	7.65	1.41	1.75

¹ 0.64 ppm endosulfan was detected on Farm No. 12 in 1969.

Trace amounts of lindane were detected on Farm No. 1 in 1966 and 1969 and on Farm No. 13 in 1966. No residues of methoxychlor were found in any of the soils.

² o,p'-DDT + p,p'-DDT.

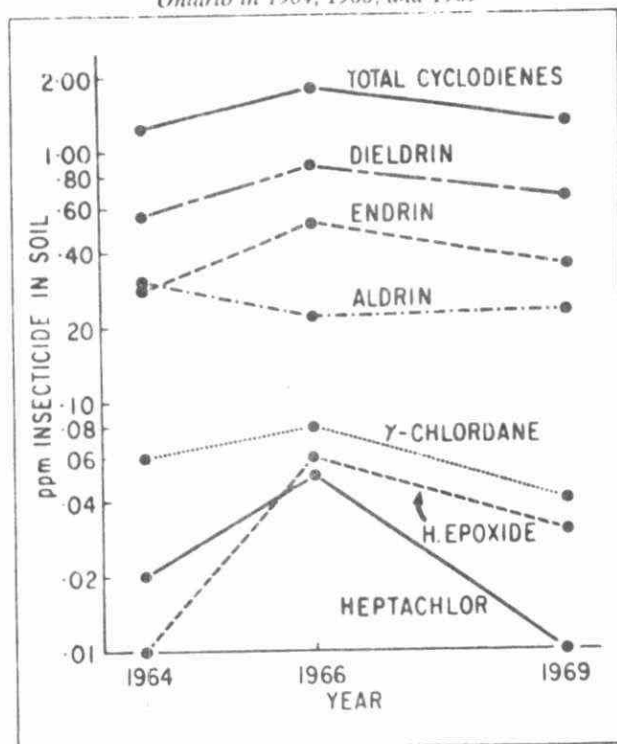
NOTE: T = trace = <0.1 ppm in 1964 and <0.01 ppm in 1966 and 1969.

— = no residue detected; limit of sensitivity 0.01 ppm in 1964; 0.001 ppm in 1966 and 1969.

residues were present on nine farms, indicating increased use of this compound as some of the other insecticides were phased out. Trace amounts of lindane were detected on two farms, but no residues of methoxychlor were found. Dicofol was present in relatively high concentrations in the two orchard soils while endosulfan was detected on Farm 12 in 1969.

For the purpose of this discussion, the results obtained on 15 of the farms have been averaged in order to point out general trends. Farm 9 was excluded since a 1969 sample was not obtainable. It should be noted, however, that due to the limited number of samples the results may not be statistically significant. As mentioned above, between 1961 and 1964 the organochlorine insecticides were used almost exclusively, but from 1965-69 increased emphasis was placed on use of the organophosphorus insecticides (Table 1). The average residue levels for the cyclodiene insecticides in soil tended to be consistent with the use pattern. In all cases, with the exception of aldrin, cyclodiene insecticide residues were highest in 1966 and appear to have declined slightly since then (Fig. 1). The highest concentration of aldrin occurred in 1964, and it was present at slightly lower levels in both 1966 and 1969. The most pronounced decline between 1966 and 1969 occurred with heptachlor. Dieldrin, endrin, γ -chlordane, and heptachlor epoxide appear to have decreased at slower, parallel

FIGURE 1.—Average residue levels (ppm) of the cyclodiene insecticides found in soil on 15 farms in southwestern Ontario in 1964, 1966, and 1969



rates. The average total cyclodiene insecticide residue levels of 1.24, 1.82 and 1.32 ppm for 1964, 1966, and 1969, respectively, indicate that the residue levels in the soil in 1969 were similar to those found in 1964.

Residues of technical DDT also reflected the changing use pattern; they were highest in 1966 (Fig. 2) and by 1969 had declined to an average value of 13.7 ppm, as compared to the 1964 level of 17.4 ppm. Residues of DDE and DDD remained relatively stable, presumably reflecting the microbial degradation of DDT to these compounds. Residues of dicofol in the two orchards sampled also showed little decrease. The average total residue levels for DDT and the related compounds were 20.17, 24.74, and 16.32 ppm for 1964, 1966, and 1969, respectively, thus indicating that residues of DDT and the related materials in 1969 had decreased to a level lower than that found in 1964.

Of the 15 farms sampled in 1964, 1966, and 1969, 4 fitted the category of field crops, 4 were tobacco farms, 5 were vegetable farms, and 2 were orchards (Table 1). When the data obtained on the cyclodiene insecticides were summarized on the basis of these four general categories (Table 3), it was apparent that vegetable soils, on the average, contained the highest levels of these compounds. Tobacco soils also contained residues of most of the common cyclodiene insecticides, but at considerably lower levels. Residues of aldrin, dieldrin, and endrin were present in the field crop soils. The average cyclodiene insecticide residues for the four field crop soils were greater than those found in tobacco soils. However, this was due primarily to the fact that on Farms 3 and 4, turnips had been included in the rotation prior to 1965, and aldrin was used for cabbage

TABLE 3.—Average residue levels for common cyclodiene insecticides found in agricultural soils on 15 farms in southwestern Ontario in 1964, 1966, and 1969 in relation to cropping practice

CROPS	NO. OF FARMS SAMPLED	YEAR	AVERAGE RESIDUE LEVELS IN PPM						
			HEPTACHLOR	HEPTACHLOR EPOXIDE	γ -CHLORDANE	ALDRIN	DIELDRIN	ENDRIN	TOTAL CYCLODienes
Field	4	1964	—	—	—	0.30	0.58	—	0.88
		1966	—	—	—	0.16	0.73	T	0.89
		1969	—	—	—	0.11	0.68	0.03	0.82
Tobacco	4	1964	T	0.04	0.07	0.06	0.35	0.03	0.55
		1966	0.11	0.10	0.08	0.04	0.41	0.07	0.81
		1969	T	0.05	0.05	0.01	0.26	0.05	0.42
Vegetable	5	1964	0.05	T	0.13	0.58	0.83	0.75	2.34
		1966	0.05	0.09	0.17	0.46	1.57	1.39	3.73
		1969	0.02	0.04	0.10	0.54	1.10	0.94	2.74
Fruit	2	1964	—	—	—	—	—	—	—
		1966	—	—	—	—	—	—	—
		1969	—	—	—	—	—	—	—

NOTE: T = trace = <0.1 ppm in 1964 and <0.01 in 1966 and 1969.
— = no residue detected.

TABLE 4.—Average residue levels of DDT, and its metabolites and dicofol found in agricultural soils on 15 farms in southwestern Ontario in 1964, 1966, and 1969 in relation to cropping practices

CROPS	NO. OF FARMS SAMPLED	YEAR	AVERAGE RESIDUE LEVELS IN PPM				
			DDT ¹	DDE	DDD	DICOFOL	TOTAL DDT RELATED COMPOUNDS
Field	4	1964	0.74	T	—	—	0.74
		1966	0.21	0.07	T	—	0.28
		1969	0.32	0.11	T	—	0.43
Tobacco	4	1964	2.86	0.16	0.04	—	3.06
		1966	3.85	0.65	0.06	—	4.56
		1969	2.69	0.63	0.06	—	3.38
Vegetable	5	1964	17.40	0.80	0.25	—	18.45
		1966	31.45	1.49	0.51	—	33.45
		1969	19.08	1.29	0.50	—	20.87
Fruit	2	1964	80.02	10.53	3.00	4.03	97.58
		1966	79.79	8.89	1.63	2.71	93.02
		1969	49.53	8.02	1.73	3.34	62.62

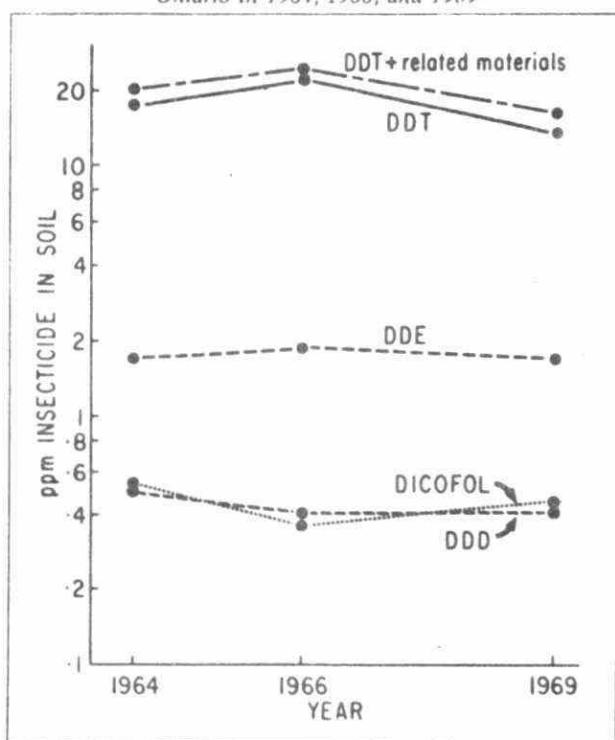
¹ o,p'-DDT + p,p'-DDT.

NOTE: T = trace = <0.1 ppm in 1964 and <0.01 ppm in 1966 and 1969.
— = no residue detected.

maggot control. The data obtained on Farms 1 and 2 are more typical of farms devoted to field crops. No residues of the cyclodiene insecticides were found in the orchards.

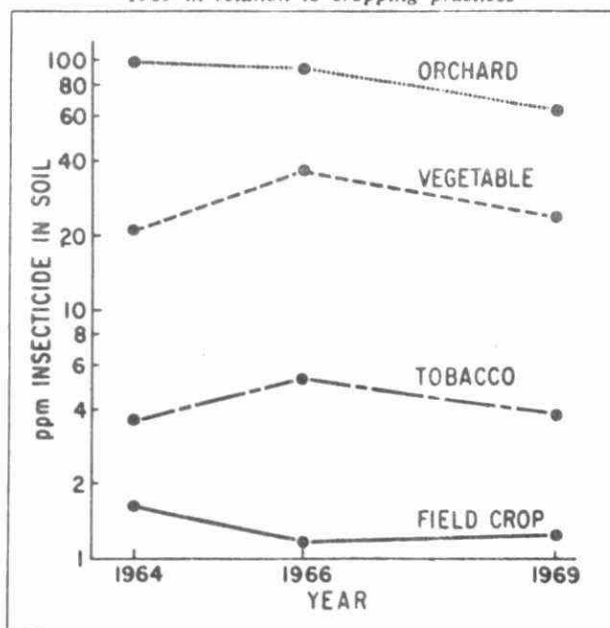
When the data for DDT were summarized in relation to cropping practices (Table 4), it was apparent that orchards contained high residues of DDT and related materials followed by vegetable, tobacco, and field crops in that order.

FIGURE 2.—Average residue levels (ppm) of DDT and related materials found in soil on 15 farms in southwestern Ontario in 1964, 1966, and 1969



Average residue levels for all the organochlorine insecticides detected in relation to cropping practices (Fig. 3) indicated that the highest residues were present in orchards > vegetable > tobacco > field crop soils. In orchards, the residue levels were highest in 1964, and decreased in both 1966 and 1969. In vegetable and tobacco soils, residues reached a peak in 1966 and decreased in 1969 to a point only slightly higher than levels found in 1964. In field crop soils, total organochlorine insecticide residues were highest in 1964 and have dropped since then.

FIGURE 3.—Average residue levels (ppm) of the organochlorine insecticides found in soil on 15 farms in southwestern Ontario in 1964, 1966, and 1969 in relation to cropping practices



The residue levels found in agricultural soils in relation to cropping practices are based on a very small number of samples. Nevertheless, the data for orchard, vegetable, and tobacco soils are probably quite representative of the situation in southwestern Ontario. However, the data on field crops are unquestionably biased. Of the four farms in this general category, two contained relatively high residues of the cyclodiene insecticides as a result of turnip production; the other two contained residues resulting from corn rootworm and seed maggot control measures. The corn rootworm is a problem only in the southernmost counties of the Province, and the acreage of turnips is limited. Consequently, the data given are not representative of the large acreage of agricultural land devoted to field crops which receive little or no insecticide treatment.

The total land area of the Province of Ontario comprises over two hundred million acres (11) (Table 5). Of this, only 6% is devoted to commercial farming operations, and over one-half of this acreage is planted in field crops where little insecticide is required. Soils containing high residue levels, i.e., tobacco, vegetable, and orchard soils, comprise 0.13% of the total land area of the Province and 2.4% of the land devoted to commercial farming. Thus, although relatively high residue levels are present in these particular soils, they are concentrated in relatively small pockets. In addition, particularly in vegetable soils, the highest residue levels were found in muck soils where they are adsorbed and their insecticidal properties inactivated; under these conditions, residues cannot be absorbed by crops and are subject to very little vertical movement (3,4,5). Nevertheless, residues of the organochlorine insecticides can move from these contaminated soils by either wind or surface water erosion to contaminate adjacent areas as well as streams and lakes.

TABLE 5.—Total acreage of land in the Province of Ontario and acreage devoted to agricultural production, 1969

	ACREAGE	PERCENT OF TOTAL
Total land area of Province	220,218,880	100.0
Commercial farms	13,229,561	6.0
Field crops (other than tobacco)	7,559,000	3.4
Tobacco	120,000	0.05
Vegetables	121,489	0.05
Fruit	77,869	0.03

The enzyme inhibition tests in 1969 indicated that inhibitory substances were generally below significant levels in field crop, tobacco, and orchard soils (Table 6). However, both the third and fourth fractions from the extracts of vegetable soils generally showed significant inhibition, thus indicating the presence of organophosphorus insecticides or their metabolites. It should be pointed out that some of the metabolites of organophosphorus insecticides have a much greater inhibitory effect than the parent materials, and therefore the data

obtained on the fourth fraction may be indicative of only minute quantities of highly inhibitive compounds. GLC analyses confirmed the presence of dichlofenthion in soils on Farms 10 and 14 in 1964, Farms 10 and 12 in 1966, and Farms 10 and 14 in 1969. Ethion was detected in the soil on Farm 13 in 1966 and 1969, diazinon on Farm 14 in 1966 and 1969, diazoxon on Farm 13 in 1969, and parathion and paraoxon on Farm 11 in 1969. Federal and provincial government regulations and recommendations have placed considerable emphasis since 1966 on decreased use of the organochlorine insecticides and increased use of the organophosphorus and carbamate insecticides. These results reflect the trend toward increased use of the organophosphorus insecticides (Table 1) in that they were detected in soils on only 2 of 5 vegetable farms in 1964, in 4 of 5 in 1969. These particular organophosphorus insecticides are generally considered to be of limited persistence in soil. However, they are often applied at higher rates and over shorter intervals during the growing season, and their limited persistence may be offset to some extent by the greater total amounts applied.

Acknowledgments

The technical assistance of H. Simmons and Miss Lucille Ho is gratefully acknowledged.

See Appendix for chemical names of compounds discussed in this paper.

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TABLE 6.—Enzymatic inhibition by extracts of agricultural soils from 16 farms in southwestern Ontario in 1969, and residues of organophosphorus insecticides detected by GLC in 1964, 1966, and 1969

FARM No.	EXTRACT FRACTION	PERCENT INHIBITION ¹ FROM EXTRACTS EQUIVALENT TO SOIL SAMPLES OF:			ORGANOPHOSPHORUS INSECTICIDES DETERMINED BY GLC (PPM)		
		0.04 g	0.4 g	1.6 g	1964	1966	1969
1	3	1.7	3.4	7.2	—	—	—
	4	5.1	14.0	40.7	—	—	—
2	3	2.5	2.1	2.5	—	—	—
	4	3.0	4.2	5.5	—	—	—
3	3	5.4	5.4	5.4	—	—	—
	4	7.8	12.2	18.1	—	—	—
4	3	0.4	2.1	5.5	—	—	—
	4	0.6	8.9	30.6	—	—	—
5	3	2.1	1.7	9.4	—	—	—
	4	3.0	2.2	9.8	—	—	—
6	3	2.6	2.6	2.1	—	—	—
	4	4.3	8.1	23.8	—	—	—
7	3	4.3	4.7	—	—	—	—
	4	4.7	8.1	—	—	—	—
8	3	2.4	3.4	8.2	—	—	—
	4	4.4	6.3	17.9	—	—	—
9	3	—	—	—	—	—	—
	4	—	—	—	—	—	—
10	3	10.8	67.9	81.7	dichlo(0.85)	dichlo(1.10)	dichlo(0.32)
	4	11.7	72.5	82.5	—	—	—
11	3	16.3	80.6	82.0	—	—	P(1.71)
	4	74.1	82.0	83.3	—	—	Po(0.01)
12	3	2.2	7.9	14.9	—	dichlo(0.03)	—
	4	8.3	42.9	74.6	—	—	—
13	3	1.7	6.0	20.0	—	ethion(0.29)	ethion(0.24)
	4	35.7	80.9	88.9	—	—	Dzo(0.03)
14	3	3.4	11.0	55.1	dichlo(0.45)	Dz(0.09)	dichlo(0.07), Dz(0.07)
	4	4.2	26.3	66.9	—	—	—
15	3	3.5	7.5	18.4	—	—	—
	4	3.5	18.0	26.3	—	—	—
16	3	1.8	16.1	16.3	—	—	—
	4	2.2	18.8	24.6	—	—	—

¹ Significant only if percent inhibition is >20.

NOTE: — = No residues detected.

Blanks = No sample available.

Limit of sensitivity: 1964 = 0.1 ppm, 1966 = 0.01 ppm, 1969 = 0.001 ppm.

dichlo = dichlofenthion

P = parathion

Dz = diazinon

Po = paraoxon

Dzo = diazoxon

Metabolism of Heptachlor and Its Degradation Products by Soil Microorganisms¹

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ABSTRACT

Chemical and soil microbial degradation of heptachlor proceeded by at least 3 pathways: epoxidation, hydrolysis, and reduction. Soil microorganisms converting heptachlor to its epoxide were identified. Thirty-five of 47 fungi, and 26 of 45 bacteria and actinomycetes isolated from soil produced the epoxide. In aqueous media, heptachlor hydrolyzed chemically to 1-hydroxychlorlone, which the

soil microorganisms were able to epoxidize to 1-hydroxy-2,3-epoxychlorlone. Heptachlor was dechlorinated by bacteria to chlorlone which was then epoxidized to chlorlone epoxide. The insecticide and its byproducts were concentrated in the fungal mycelium. The presence of cyclodiene insecticides in the media appeared to influence some microbial metabolic processes.

The influence of microorganisms on the degradation of pesticides in soils is being investigated by an increasing number of researchers. Lichtenstein and Schulz (1960, 1964), Getzin and Rosefield (1968), and Bro-Rasmussen et al. (1968) compared the persistence of pesticides in sterilized and nonsterilized soils. Persistence was less, and conversion to other products was greater, in the nonsterilized soils, and the differences were attributed to the activity of microorganisms. Weber and Coble (1968) demonstrated the decomposition of diquat by the microbes in an aqueous soil extract. Cserjesi (1967), Perry and Scheld (1968), Poonawalla and Korte (1968), Matsumura and Boush (1968), Matsumura et al. (1968), Tu et al. (1968), and Gunner and Zuckerman (1968) examined the action of specific microorganisms on pesticides and related hydrocarbons.

Heptachlor, first isolated from technical chlorlone, has been an important soil insecticide since 1949. Davidow and Radomski (1953), Davidow et al. (1953), Radomski and Davidow (1953), and Harris et al. (1956) reported the conversion of heptachlor to heptachlor epoxide in animals. Conversion to the epoxide on plants was demonstrated by Gannon and Decker (1958) and in soil by Gannon and Bigger (1958) and Lichtenstein and Schulz (1960). Although it has been assumed that soil microbes are responsible for the conversion of heptachlor to its epoxide in soil, the specific organisms involved had not been determined. Hydrolysis of heptachlor to 1-hydroxychlorlone has

been shown to occur in aqueous suspensions by Bowman et al. (1964), and there is some evidence that this conversion occurs also in soils (Bowman et al. 1965, Duffy and Wong 1967).

This study was initiated to isolate and identify the microorganisms converting heptachlor to its epoxide in soil and to determine if there were other pathways of chemical and microbial degradation of heptachlor.

METHODS AND MATERIALS.—The isolation of the 92 species of microorganisms used in this study from soil was described previously (Tu et al. 1968). Tables 1 and 2 list the genera. Heptachlor and/or its metabolites were incubated at 28°C with each species in an aqueous basal medium. The insecticide was added in ethanol solution to give a concentration of 1 ppm and 1% ethanol. Composition of the medium was: KH_2PO_4 , 1 g; K_2HPO_4 , 1 g; NH_4NO_3 , 1 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 g; CaCl_2 , 0.02 g; $\text{Fe}_2(\text{SO}_4)_3$, 0.01 g; and distilled water to make 1 liter. The pH was adjusted to 7.0. Additions of insecticide were made at zero day, 3, 4, and 5 weeks, and analyses were conducted 6 weeks after the 1st addition. Products were extracted from the medium with hexane. The hexane extract was dried with anhydrous sodium sulfate and analyzed by GLC using electron capture detectors. Columns were 1.5 m long and 3 mm o.d. Column packings were: 5% DC 200 (methyl silicone) operated at 185°C; 3% OV-17 (methyl phenyl silicone), 182°C; 5% XE-60 (nitrile silicone), 200°C; and 5% QF-1 (fluorosilicone), 185°C. Solid support was 100/120 mesh Aeropak 30, except for OV-17 which was coated on 60/80 mesh Gas Chrom Q. Identities of products

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were established by retention times on these 4 liquid phases compared with authentic reference standards. Identities were confirmed by TLC and chemical conversion to known compounds.

Early analyses of fungal samples showed a remarkable sorption of heptachlor and heptachlor epoxide by the mycelium. In all subsequent analyses of fungi, the mycelia were separated by filtration, ground with celite, dried with anhydrous sodium sulfate, and extracted with hexane while subjected to ultrasonic vibrations at 80 Kh. The filtrates were extracted in separatory funnels with hexane.

RESULTS AND DISCUSSION.—Our analyses showed the formation of the following products from heptachlor: 1-hydroxychlorde, heptachlor epoxide, chlordene, chlordene epoxide, 1-hydroxy-2,3-epoxychlorde, and 1 unknown.

Fig. 1 shows a schematic diagram for the production of these materials from heptachlor. Table 1 details the results of the fungal incubations. Table 2 shows the results of the incubations with actinomycetes and bacteria. Of 47 fungi, 35 produced heptachlor epoxide in amounts varying from 0.001 to 0.175 ppm. Of 45 bacteria, 26 produced heptachlor epoxide (0.001 to 0.241 ppm). Conversion of heptachlor to its epoxide was not so great as the 9% found in

the work on the conversion of aldrin to dieldrin (Tu et al. 1968), which is understandable considering the alternative degradation routes open to heptachlor. The greatest conversion to heptachlor epoxide was by a *Nocardia* species which converted 6% of the applied heptachlor to heptachlor epoxide in 6 weeks. Production of heptachlor epoxide was not confined to any single genus. Heptachlor epoxide was produced by cultures of *Rhizopus*, *Fusarium*, *Penicillium*, *Trichoderma*, *Nocardia*, *Streptomyces*, *Bacillus*, and a *Micromonospora*. Repeat incubations did not always result in the same level of epoxide production. In 1 experiment, a *Nocardia* culture converted 6% of the heptachlor to its epoxide in 6 weeks, but converted only 2% in the experiment detailed in Table 2. This lack of quantitative duplication may be caused by either physical or biological variations. Volatilization of heptachlor from the medium would reduce the amount available for epoxidation. Recent experiments at this laboratory indicate that adsorption onto the glass walls of test tubes and flasks can quickly deplete the concentration of insecticide in aqueous solution even with an ethanol content of 1%. Biological variations might occur within the microbial cultures from one test to the next, especially with *Actinomyces* (Kuster 1967). While efforts were main-

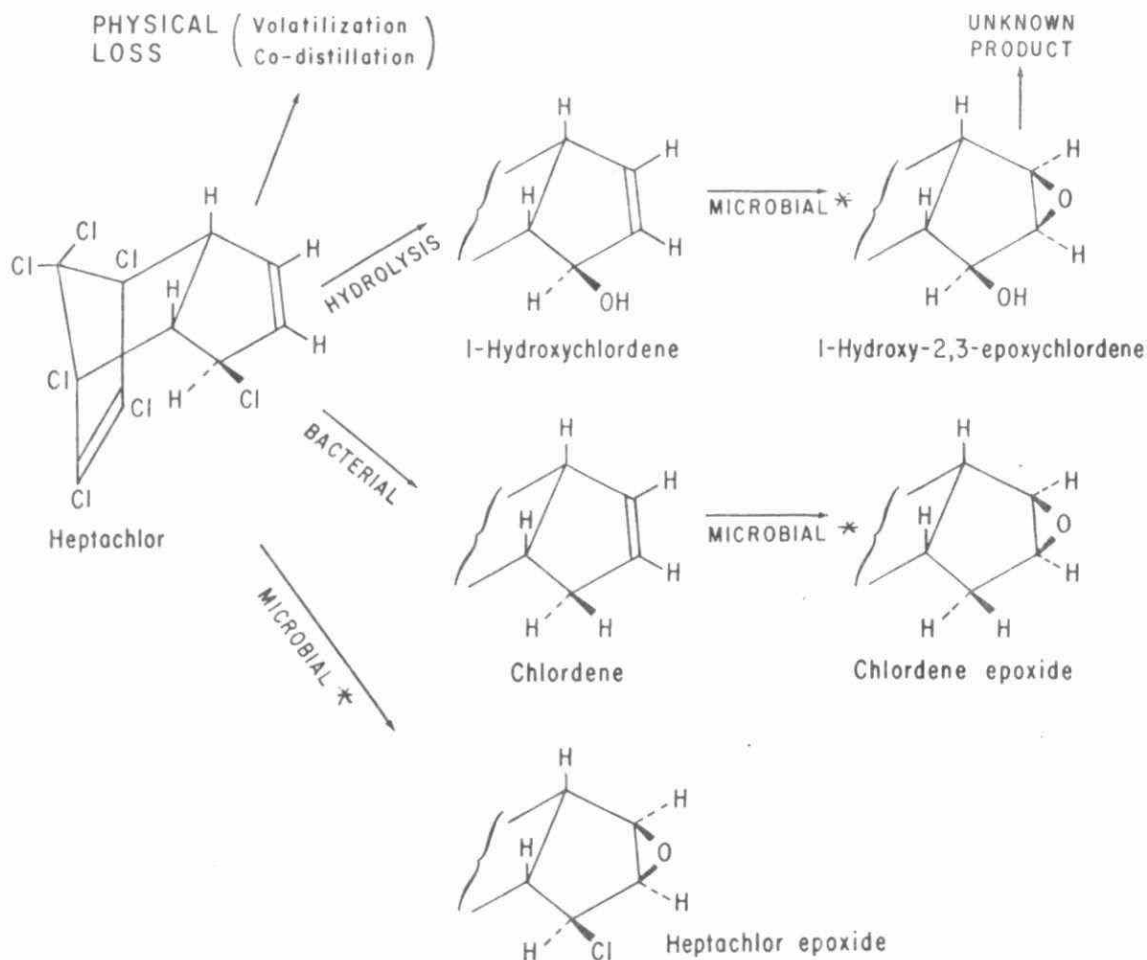


FIG. 1.—Scheme for chemical and microbial degradation of heptachlor.

Table 1.—Fungal conversion of heptachlor.*

Genus and no. species	Heptachlor epoxide ^b ppm	1-hydroxy-chlordene ^c ppm	1-hydroxy-2,3-epoxy-chlordene ^b ppm
<i>Trichoderma</i> (15)	0.000 to 0.014	0.10 to 0.50	<0.01 to 0.10
<i>Penicillium</i> (11)	.000 to .048	.06 to .41	< .01 to .09
<i>Fusarium</i> (16)	.000 to .082	.07 to .62	< .01 to .25
<i>Aspergillus</i> (2)	.000	.11	<.01
	.001	.21	<.01
<i>Rhizopus</i> (2)	.013	.41	.12
	.175	.15	.03
<i>Mucor</i> (1)	.002	.61	.01
Control (2)	.000	1.00	.00
	.000	.99	.00

* Heptachlor was added to the medium at 1 ppm on zero day, and at 3, 4 and 5 weeks for a total of 4 ppm. Samples were analyzed 6 weeks after the 1st addition.

^b Heptachlor epoxide and 1-hydroxy-2,3-epoxy-chlordene were detected only on the mycelium.

^c 1-hydroxy-chlordene was partly sorbed on the mycelium, the remainder was in solution in the medium—the figures given are the totals.

tained to minimize these variations, we did not always achieve quantitative duplication. However, qualitative duplication was always obtained.

Although no 1-hydroxy-chlordene was present in the analytical grade heptachlor added to the media, it occurred in almost all samples after incubation. The greatest amounts were in the control samples which contained heptachlor but no microorganisms, therefore the 1-hydroxy-chlordene was produced by chemical rather than by microbial action. Amounts of 1-hydroxy-chlordene in the samples (Tables 1, 2) range downward from the 1.00 and 1.20 ppm found in the controls. Four of the 45 bacteria and actinomycetes produced 1-hydroxy-2,3-epoxy-chlordene in concentrations from 0.14 to 0.46 ppm. By contrast 43 of 47 fungi produced this degradation product in amounts ranging from a trace to 0.25 ppm.

1-hydroxy-2,3-epoxy-chlordene could conceivably be produced by epoxidation of 1-hydroxy-chlordene at the 2,3 positions, or by hydrolysis of heptachlor epoxide at the no. 1 carbon. To determine the source of the 1-hydroxy-2,3-epoxy-chlordene, we incubated 1-hydroxy-chlordene and heptachlor epoxide separately with specially selected bacteria for periods up to 10 weeks. The 1-hydroxy-2,3-epoxy-chlordene was produced only from 1-hydroxy-chlordene. In this experiment the *Micromonospora* spp. totally converted 1 ppm of 1-hydroxy-chlordene to 1-hydroxy-2,3-epoxy-chlordene (measured as 1.1 ppm) and a small unknown peak. 1-hydroxy-2,3-epoxy-chlordene has been reported by Klein et al. (1968) to result from animal metabolism of heptachlor, and by Brooks and Harrison (1965) from house fly, *Musca domestica* L., metabolism of chlordene, but has not previously been reported as a product of soil microbial action.

The incubation of 1-hydroxy-chlordene with a *Nocardia* spp. and the *Micromonospora* spp. separately has produced an unknown peak ($R_t = 0.8 \times$ 1-hydroxy-chlordene time on XE-60) besides producing 1-hydroxy-2,3-epoxy-chlordene. We theorized that the unknown might be 1-ketochlordene, and in fact the R_t of the unknown agrees with that of 1-ketochlordene on 3 of our GLC liquid phases. Cochrane² sup-

plied us with the standard 1-ketochlordene and postulated that the 1-ketochlordene could result from dehydration on GLC columns of rearranged 1-hydroxy-2,3-epoxy-chlordene. The source and identity of the unknown is presently being investigated.

Table 2 shows 18 actinomycetes and bacteria produced chlordene (up to 0.364 ppm), which had not previously been reported as a degradation product of heptachlor. Since heptachlor is produced commercially by the chlorination of chlordene, contamination by chlordene might be expected, but our GLC analyses showed no trace of chlordene in the heptachlor standards or control samples. The identity of the chlordene product was verified on all 4 GLC columns and further confirmed by bromination of test samples and standard chlordene and reexamination by GLC for 1-bromochlordene. Chlordene could conceivably result from dechlorination of heptachlor or by removal of the hydroxyl from the 1-hydroxy-chlordene present in the solution as a result of hydrolysis of the heptachlor. To determine if chlordene was produced from 1-hydroxy-chlordene, we selected the bacteria which had produced the greatest amounts of chlordene, and incubated them with 1-hydroxy-chlordene. No chlordene was produced, and we therefore concluded that the chlordene was derived by dechlorination of heptachlor. A parallel to this dechlorination of heptachlor exists in the conversion of DDT to DDD (Kallman and Andrews 1963, Plimmer et al. 1968), but this is a reduction of an ethane carbon, while with heptachlor the reduction is of the allylic carbon of a cyclopentene. To confirm our results we again incubated heptachlor for 6 weeks with the bacteria. Chlordene was produced, but not in the same amounts as before, indicating a qualitative but not a quantitative duplication. When chlordene was incubated at 1 ppm in aqueous medium with selected oxidizing bacteria, chlordene epoxide was produced, but in very minor quantities.

Gunner and Zuckerman (1968) showed synergistic

Table 2.—Actinomycetes and bacterial conversion of heptachlor.*

Genus and no. species	Heptachlor epoxide ppm	Chlordene ppm	1-hydroxy-chlordene ppm	1-hydroxy-2,3-epoxy-chlordene ppm
<i>Nocardia</i> (16)	0.000 to 0.086 ^b	0.000 to 0.044	0.34 to 1.26	0.00 to 0.14
<i>Streptomyces</i> (7)	.000 to .055	.000 to .035	.37 to 1.26	all .00
<i>Thermoactinomyces</i> (3)	.000 to .004	all .000	.71 to .88	all .00
<i>Micromonospora</i> (1)	.049	.001	1.26	.20
<i>Bacillus</i> (12)	.000 to .049	.000 to .364	.23 to 1.26	.00 to .46
<i>Arthrobacter</i> (5)	.000 to .003	.000 to .010	.42 to 1.17	all .00
<i>Corynebacterium</i> (1)	.001	.000	.98	.00
Controls (2)	.000	.000	1.20	.00
	.000	.000	1.17	.00

* Heptachlor was added to the medium at 1 ppm on zero day, and at 3, 4 and 5 weeks for a total of 4 ppm. Samples were analyzed 6 weeks after the 1st addition. Cells were not separated. Cells and medium were extracted together.

^b *Nocardia* culture which produced 0.086 ppm heptachlor epoxide, produced 0.241 ppm in a later incubation.

² Dr. W. P. Cochrane, Production and Marketing Branch, Can. Dep. Agr., Ottawa, Ontario. Personal communication (1968).

microbial action between an *Arthrobacter* spp. and a *Streptomyces* species which resulted in the complete degradation of diazinon in 21 days. When the microorganisms were incubated separately no change was evident in the diazinon molecule. Our experiments have doubtless oversimplified the action of soil microbes, since we have incubated in vitro individual species of soil microbes with heptachlor and its degradation products whereas in soil or in a water system the microorganisms probably act in combination. It is quite possible that some of the cultures which showed little or no activity in our study might be powerful converters if incubated in various combinations.

This report shows that the soil microorganisms effect considerable changes in the insecticides and their metabolites. The effect of insecticides on microorganisms must also be examined. Ko and Lockwood (1968) found that DDT at concentrations as low as 1 or 10 ppm was highly toxic to soil bacteria and actinomycetes in culture, but found little effect on total microbial members in soil by DDT and DDD. Studies which we have conducted (unpublished data) indicate that cyclodiene insecticides do not have any pronounced influence on total members of the various species in the soil. However, some of our gas chromatographic analyses of fungal incubations indicate that the insecticides may have some effect on the metabolism of microorganisms. The dotted curve of Fig. 2 shows a strong electron capturing peak obtained from extracts of a *Trichoderma* fungus. This peak is absent in the curve for fungus incubated with heptachlor (solid curve). We have similar evidence of the suppression of metabolic products of another *Trichoderma* by aldrin. These data indicate a definite influence of the cyclodiene insecticides on the metabolic products of the fungi and suggest that future work should consider the action of insecticides

on microbial metabolism as well as the action of microorganisms on insecticides.

Chacko and Lockwood (1967) described the accumulation of DDT and dieldrin by microorganisms. As just mentioned, our techniques were modified so that products sorbed on the fungal mycelium could be analyzed separately from products in solution. The degree of sorption by the mycelium is indicated by a *Rhizopus* which had converted 4.4% of the applied heptachlor to heptachlor epoxide in 6 weeks. The mycelium contained 95% of the total chemicals (heptachlor and its degradation products), only 5% being in solution in the medium.

In the past, it has been assumed that the degradation of heptachlor in the environment followed 2 major pathways, i.e., volatilization of the parent material from soil, plants, or water into the air, or conversion to heptachlor epoxide. It is apparent from this study that there are 2 other pathways of degradation (Fig. 1), i.e., chemical hydrolysis to 1-hydroxychlorde followed by microbial epoxidation to 1-hydroxy-2,3-epoxychlorde and conversion to an unknown product; and bacterial dechlorination of heptachlor to chlorde and then oxidation to chlorde epoxide. The former appears to be a major degradation route, the latter of lesser importance. The significance of these results remains to be determined. However, 1-hydroxychlorde has been reported as a residue in soil resulting from heptachlor treatments in at least 2 instances (Bowman et al. 1965, Duffy and Wong 1967). Preliminary laboratory studies which we have conducted indicate that the production of 1-hydroxychlorde in soil is comparable to that of heptachlor epoxide. It is conceivable that, in instances where heptachlor residues in soil are transported into water, the 1-hydroxychlorde route of degradation may assume a greater degree of importance. Brooks and Harrison (1965) reported that 1-hydroxychlorde and 1-hydroxy-2,3-epoxychlorde were non-toxic when injected into house flies. Although we have not been able to locate data on the mammalian toxicity of the compounds produced in the hydroxychlorde pathway of degradation, it seems likely that these products will be less toxic than either the parent material or heptachlor epoxide.

ACKNOWLEDGMENTS.—The technical assistance of Mrs. Margaret Thomson and Mr. W. W. Sans is gratefully acknowledged. The reference standards used in identifying the heptachlor metabolites in this study were provided as follows: chlorde epoxide, 1-hydroxy-2,3-epoxychlorde, and 1-ketochlorde, Dr. W. P. Cochrane, Production and Marketing Branch, Canada Department of Agriculture, Ottawa; heptachlor, chlorde, 1-hydroxychlorde, 1-bromochlorde, and heptachlor epoxide, Velsicol Corp., Chicago, Ill.

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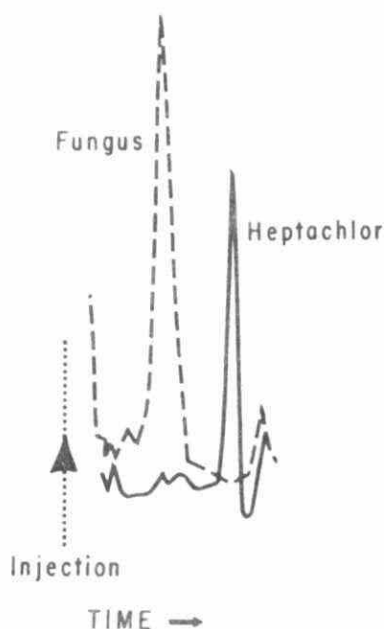


FIG. 2.—Electron capture gas chromatographic response from a *Trichoderma* spp., and *Trichoderma* spp. incubated with heptachlor.

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Degradation of Heptachlor Epoxide and Heptachlor by a Mixed Culture of Soil Microorganisms¹

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ABSTRACT

A mixed culture of soil microorganisms, obtained from a sandy loam soil, degraded heptachlor epoxide to the less toxic 1-exohydroxychlorde. Conversion was about 1% per week during the 12-week test periods. The same mixed culture reduced heptachlor to chlordene but was inactive when incubated with 1-exohydroxychlorde or

1-exohydroxy-2,3-epoxychlorde.

This degradation of heptachlor epoxide may explain the occurrence of high levels of 1-exohydroxychlorde and low levels of heptachlor epoxide found in heptachlor-treated soils.

Incubation of the insecticide heptachlor with 92 pure cultures isolated from a sandy loam soil has been reported (Miles et al. 1969). In that study, heptachlor was reduced by bacteria to chlordene, a previously unreported reaction, and hydrolyzed by the aqueous medium to 1-exohydroxychlorde. Both bacteria and fungi oxidized heptachlor to heptachlor epoxide, chlordene to chlordene epoxide, and 1-exohydroxychlorde to 1-exohydroxy-2,3-epoxychlorde. Incubation of insecticidal substrates with pure soil microbial cultures in vitro shows the action of the specific microorganisms, but the results may not indicate the fate of insecticidal residues in vivo, where the combined action of the soil microorganisms is doubtless influenced by antagonism and synergism.

This paper reports on incubations of heptachlor, heptachlor epoxide, 1-exohydroxychlorde, and 1-exohydroxy-2,3-epoxychlorde with a mixed culture of soil microorganisms extracted from a sandy loam soil by water.

MATERIALS AND METHODS.—Incubations in Test Tubes.—Twenty g of sandy loam soil were shaken with 100 ml of sterile distilled water. The sand was allowed to settle for 10 sec, then 1-ml aliquots of the supernatant suspension were withdrawn and transferred to test tubes containing 12 ml of aqueous

medium consisting of the pesticide substrate and 1% ethanol. Heptachlor, heptachlor epoxide, 1-exohydroxychlorde, and 1-exohydroxy-2,3-epoxychlorde were tested at 1 ppm concentration. The composition of the medium was described previously (Miles et al. 1969). Stainless-steel caps were placed on the test tubes which were then incubated at 28°C for up to 12 weeks. Heptachlor, which disappears rapidly from aqueous solutions, was fortified at 1 ppm every 2 weeks. At 2-week intervals, test tubes for each treatment were removed and the contents were subjected to ultrasonic vibrations at 20 kHz from a Biosonic III (Bronwill Scientific, Rochester, N.Y.), to disrupt the cells prior to extraction. Separate test tubes containing only medium and inoculant were assayed as blanks, and others containing only medium and insecticidal substrate were assayed as a control on the degradation of insecticide in the medium alone. Extraction, GLC analysis and GLC parameters were described previously (Miles et al. 1969).

Incubations in Flasks.—To 500 ml of sterile medium in 1-liter flasks were added 5 ml of 0.0105% wt/v heptachlor epoxide in ethanol. The inoculant of mixed culture was obtained by shaking 100 g of sandy loam soil with 200 ml of distilled water, allowing the sand to settle for 10 sec, withdrawing 20 ml of the supernatant, and adding it to the liter flasks. The flask necks were closed with cotton and wrapped with aluminum foil. The flasks were incu-

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bated at 28°C. The test was run in quadruplicate flasks; a 5th flask was not treated with heptachlor epoxide and served as a medium and inoculant blank.

After 4, 8, and 12 weeks' incubation, the flasks were shaken, and a 15-ml portion was withdrawn and subjected to ultrasonic vibration at 20 kHz for 3 min. A 10-ml aliquot of the disrupted sample was analyzed as previously described (Miles et al. 1969). Extra tests showed that the ultrasonic treatment did not increase the recovery of the insecticide or metabolite, but without this treatment the thick curds made accurate pipetting impossible.

RESULTS AND DISCUSSION.—Fig. 1 shows the products obtained from incubations of heptachlor and heptachlor epoxide with the mixed culture of soil microorganisms. Table 1 shows percent conversions of heptachlor to chlordene, 1-exohydroxychlordene, heptachlor epoxide, and chlordene epoxide. Experiments at this laboratory showed that heptachlor disappears rapidly from aqueous solutions. While this property makes quantitation of chlordene production difficult, the data in column 1 of Table 1 indicate that a fairly uniform production of chlordene, by the reduction of heptachlor, was maintained during

the 12-week sampling period. The absence of any heptachlor epoxide or chlordene epoxide products until the 12th week indicates that reducing conditions prevailed until that time. Separate incubations of 1-exohydroxychlordene with the mixed culture showed no conversion to its epoxide (1-exohydroxy-2,3-epoxychlordene) until the 10th week. No conversion of 1-exohydroxy-2,3-epoxychlordene was noted during the 12 weeks that this metabolite was incubated with the mixed culture.

Table 2 shows percent conversion of heptachlor epoxide to 1-exohydroxychlordene, and the values are from 3 separate consecutive experiments. Columns 1 and 2 list results of incubations in test tubes; column 3 lists results of incubations in liter flasks. The production of 1-exohydroxychlordene from heptachlor epoxide in the test tubes was neither so great nor so uniform as in the flasks, where conversion averaged ca. 1%/week.

The degradation reaction of heptachlor epoxide to 1-exohydroxychlordene was not reported previously. Identity of the 1-exohydroxychlordene product was established by comparison with an authentic reference standard on DC 200, XE60, and QF1, GLC columns. Identity was confirmed by mild oxidation

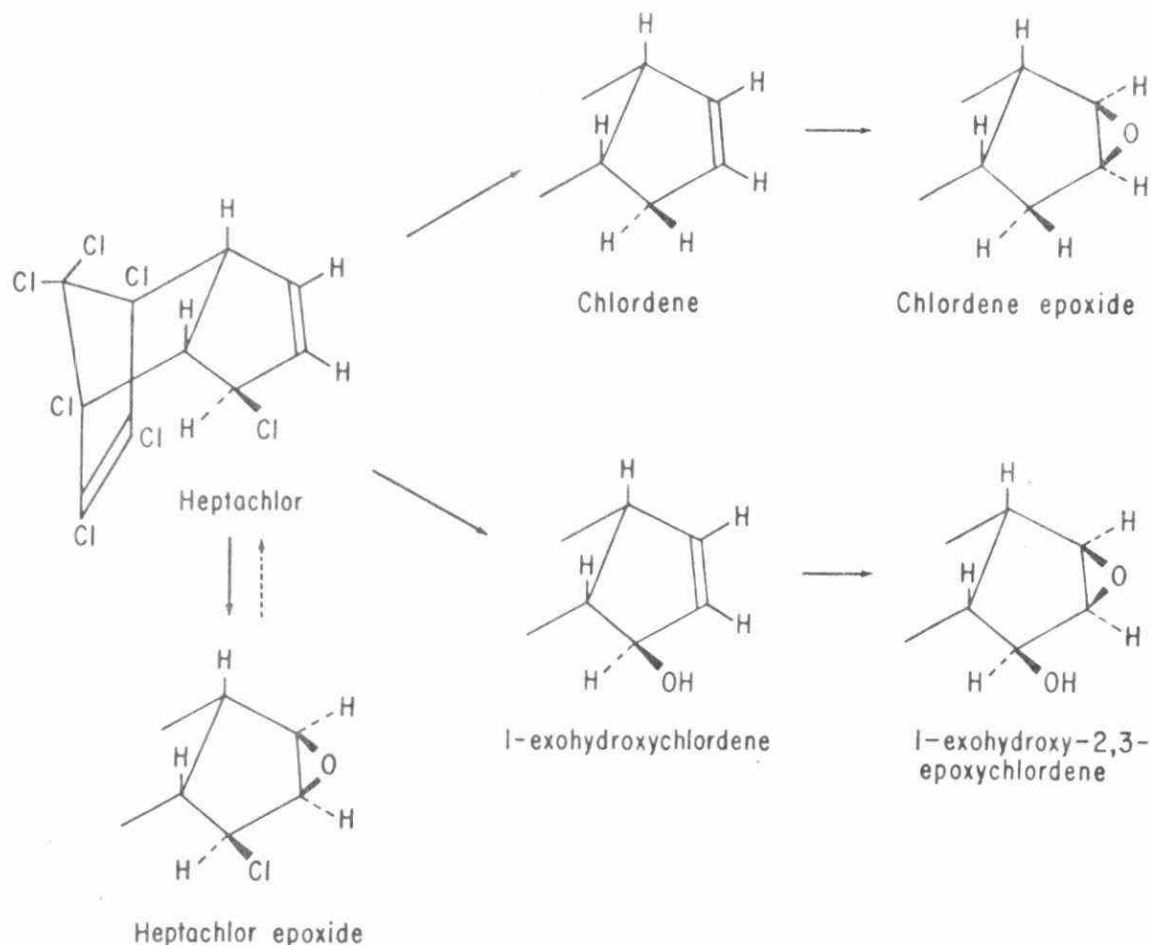


FIG. 1.—Scheme for heptachlor and heptachlor epoxide degradation by soil microorganisms. (Dotted arrow indicates possible mechanism for heptachlor epoxide degradation to 1-exohydroxychlordene (see text)).

of the extracts with chromic oxide (Starratt 1968) which converted the 1-exohydroxychloridene to 1-ketochloridene. Additional confirmation was obtained by silylation of the extracts with Regisil[®], (bis(trimethylsilyl)trifluoroacetamide) (Chemical Research Services, Addison, Ill.). The silylated products had the same retention time as that of silylated standard 1-exohydroxychloridene ($RT = 0.21$ of the retention time of the unsilylated compound on XE60 GLC column). The extracts from heptachlor epoxide incubation with the mixed culture were examined for the possible occurrence of 1-endohydroxychloridene, but no trace of the "endo" isomer was found.

1-hydroxychloridene was reported to occur as a residue in heptachlor-treated soils by Bowman et al. (1965) and Duffy and Wong (1967). Bowman et al. (1964) and Miles et al. (1969) reported that 1-exohydroxychloridene results from hydrolysis of heptachlor, and its occurrence in heptachlor-treated soils might be assumed to result solely from hydrolysis of the applied heptachlor. This study indicates that the 1-exohydroxychloridene residue may also result in part from reductive degradation of heptachlor epoxide, previously thought to be a very refractory soil pesticidal residue. Agreement with this concept can be found in a recent study of heptachlor-treated soils by Carter and Stringer (1970). They examined soils from 5 areas of the United States and found the main residue to be 1-hydroxychloridene (up to 60%). Generally, heptachlor epoxide represented only a small fraction of the residue in the soil.

Acute oral LD_{50} of 1-exohydroxychloridene to rats is 2400–4600 mg/kg so both the hydrolysis of heptachlor (LD_{50} 90–135 mg/kg) and the degradation of heptachlor epoxide (LD_{50} 60 mg/kg) are detoxication reactions (Polen²).

The mechanism for the degradation of heptachlor epoxide to 1-exohydroxychloridene was not determined. Reduction to heptachlor followed by hydrolysis is a possibility, but no heptachlor intermediate was detected in any of our extracts. However, a

² P. B. Polen, Velsicol Corp., Chicago, Ill. Personal communication.

Table 1.—Conversion of heptachlor by a mixed culture of soil microorganisms.

Incubation time (weeks)	Chloridene % ^a	1-exohydroxychloridene % ^a	heptachlor epoxide % ^a	chloridene epoxide % ^a
2	7	4	<0.04	<0.02
4	10	7	<.04	<.02
6	13	4	<.04	<.02
8	8	3	<.04	<.02
10	9.5	3	<.04	<.02
12	11	1	.40	.40

^a % Conversion based on added heptachlor. (Heptachlor was added to the medium at 1 ppm every 2 weeks.)

Table 2.—Conversion of heptachlor epoxide to 1-exohydroxychloridene by a mixed culture of soil microorganisms. (Percent.)

Incubation time (weeks)	In test tubes	In 1-liter flasks			
		A	B	C	D
2	0.6				
4	1.1	2.0	3.7	3.1	2.4
6	2.1 1.0, 2.0				
8	4.0 2.3, 6.0	4.8	6.1	5.1	7.0
10	4.3 2.3, 7.6				
12	6.1 1.0, 6.6	11.5	15.0	8.5	12.8

^a Based on added heptachlor epoxide. Heptachlor epoxide added initially at 1.0 ppm. No further additions were made.

significant quantity of chloridene, representing 5% of the initial heptachlor epoxide, was detected in one of the flask incubations at the 12th week. The presence of chloridene could be due to reduction of a portion of the heptachlor intermediate to chloridene, before hydrolysis could convert the heptachlor to 1-exohydroxychloridene.

ACKNOWLEDGMENT.—Extractions and GLC analyses were conducted by Mrs. M. H. H. Thomson. Mr. G. Hietkamp assisted in setting up the heptachlor epoxide flask incubations. GLC verification of the chloridene product was performed by Mr. W. W. Sans. Reference standards used in this study were provided as follows: chloridene epoxide, 1-hydroxy-2,3-epoxychloridene, 1-ketochloridene, and 1-endohydroxychloridene. Dr. W. P. Cochrane, Production and Marketing Branch, Canada Department of Agriculture, Ottawa; heptachlor, chloridene, 1-exohydroxychloridene and heptachlor epoxide, Velsicol Corp., Chicago, Ill.

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THE PERSISTENCE OF CERTAIN PESTICIDES IN THE SOIL AND THEIR EFFECT ON CROP YIELDS¹

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ABSTRACT

From 1954 to 1958 at Kentville, Nova Scotia, the persistence and residual effects of certain pesticides, added to the soil annually from 1949 to 1953 inclusive, were investigated. Stability of pesticides in the soil rated in descending order of persistency was as follows: arsenic, DDT, BHC, chlordane. Arsenic, DDT, and sulphur caused decreases in the yields of some crops. Evidence indicated that DDT, BHC, and parathion were translocated to root crops. Increased concentrations of arsenic in the soil resulted in increased accumulations of the element in plants.

The use of lime in the soil did not ameliorate the toxic conditions resulting from the arsenic and DDT treatments, but did correct the effects of sulphur applications.

INTRODUCTION

The effects of repeated soil applications of seven pesticides, commonly used in orchard sprays, were studied from 1949 to 1953 in a field experiment at Kentville, Nova Scotia (3). The persistence of the pesticides in the soil and their effect on the growth of various crops was investigated during the subsequent 5-year period. Results obtained are given in the present paper.

MATERIALS AND METHODS

The experimental layout was a randomized block design with four replications. The individual plots were 13 feet \times 16 feet and were separated by 5-foot cultivated strips. The soil in the experimental area has been classified as Berwick sandy loam (7), which is one of the common soil types in the Annapolis Valley. Details of the pesticide treatments, applied from 1949 to 1953, are given in Table 1.

Beans were grown annually from 1954 to 1958 inclusive. In addition turnips were grown in 1954, carrots in 1955, tomatoes in 1957, and peas in 1958.

Roots of the carrot crop grown in 1955, and seeds, pods, and vines of the bean and pea crops grown in 1958 were analysed for pesticide residues.

Prior to cultivation, a 6-12-6 fertilizer was applied each spring from 1954 to 1958 at 700 pounds, 900 pounds, 1500 pounds, 1000 pounds, and 1000 pounds per acre respectively. Dolomitic limestone, applied at 2.5 tons per acre in the fall of 1956, and at 1 ton per acre in the fall of 1957, was thoroughly mixed with the soil to a depth of 6 inches by rototilling.

Composite soil samples representative of the 0-6 inch depth were taken from each plot in the spring before planting and again at the end of each growing season. The following determinations were made: pesticide concentrations, soil reaction, content of exchangeable cations (calcium, magnesium, potassium), and available phosphorus.

Procedures followed in the analyses of soil and plant samples were the same as previously described (3).

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RESULTS AND DISCUSSION

Persistence of Pesticides in the Soil

The soil concentrations of the pesticides are given in Table 2.

The arsenic content of the soil remained relatively constant during the 5 years of the investigation. The somewhat variable results shown for arsenic concentrations in the soil (Table 2) are attributed to soil sampling technique and to the method of analysis.

DDT, BHC, and chlordane appeared to be relatively stable in the soil, although there was a gradual decrease in concentration of these compounds in successive years. Allen *et al.* (2) reported that plots treated with 100 pounds of DDT per acre in 1947 had a residue of 28.2 pounds per acre in 1951, and that after 3 years about one-half of a 100 lb.-per-acre application of BHC had disappeared. Fleming and Maines (4) reported a loss of DDT of about 10 per cent per year over a period of 8 years. Lichenstein (8), in a study involving 14 orchards, reported a recovery of 26.6 per cent of the total amount of DDT applied as sprays during a 10-year period. Losses observed in the present 5-year study indicate an annual average loss of DDT of 12 per cent, and of BHC of 16 per cent.

Previous findings (3) indicated that parathion disappears rapidly from the soil. However, in this study it was found that minute traces of the compound or related compounds, as determined by the method followed for the determination of parathion, persisted in the soil for at least 5 years.

TABLE 1.—SOIL TREATMENTS

Pesticide	Annual application (lb./ac.)	Years of application
Lead arsenate (PbHAsO_4)	419	1949, 1950, 1951, 1952, 1953
DDT (50% W.P.)	209	1949, 1950, 1951, 1952, 1953
Parathion (15% W.P.)	209	1949, 1950, 1951, 1952, 1953
Sulphur (S)	1,256	1949, 1950, —, —, —
Ferbam (70% active ingredient)	209	1949, 1950, 1951, 1952, 1953
BHC (50% W.P., 6% gamma)	52	—, 1950, 1951, 1952, 1953
Chlordane (40% W.P.)	25	—, —, 1951, 1952, 1953

TABLE 2.—AVERAGE VALUES FOR SPRING AND FALL CONCENTRATIONS OF PESTICIDES IN SOIL (RESULTS IN P.P.M.—AIR DRY BASIS)

Year of analysis	Arsenic(As)	DDT	Parathion	BHC	Chlordane
1954	126	136	0.43	10.8	1.80
1955	157	126	0.29	10.3	1.01
1956	149	114	0.29	7.9	1.43
1957	151	96	0.14	5.3	0.45
1958	127	76	0.09	5.1	0.34

TABLE 3.—EFFECT OF PESTICIDES ON CROP YIELDS
(AVERAGE YIELDS OF FOUR REPLICATES—POUNDS PER PLOT)

Crop	Year	Pesticides applied 1949–1953							Check
		Arsenic	DDT	Parathion	Sulphur	Ferbam	BHC	Chlor- dane	
Beans	1954	4.3**	2.2**	7.0	5.3*	6.8	5.9	6.5	6.8
	1955	1.8**	1.4**	5.0	2.5**	4.3	4.7	4.1	4.6
	1956	1.4**	1.5**	3.4	1.4**	3.4	3.7	3.0	3.1
	1957	3.0**	2.3**	7.7	7.7	7.9	7.9	7.7	8.0
	1958	2.5**	0.9**	5.6	5.6	5.1	5.4	5.5	4.8
Turnips	1954	36.5**	28.0	26.1	13.5	34.1**	35.5**	24.0	20.6
Carrots	1955	9.7**	8.5**	11.7	0.0**	12.5	11.4	14.1	14.1
Tomatoes	1957	96.1**	22.2**	131.4	125.4	135.1	122.5	131.7	127.1
Peas	1958	3.2**	4.3**	6.6	7.2	6.8	6.7	6.0	6.4

*Differ significantly from check at $P = 0.05$ **Differ significantly from check at $P = 0.01$ *Crop Yields*

The average yields are presented in Table 3.

Yields of the test crops, with the exception of turnips in 1954, were lower on the arsenic- and DDT-treated plots. The significantly higher yield of turnips on some plots was probably not due to a direct effect of the treatments. The chemicals may have reduced the turnip maggot infestation, although this was not definitely established. Foster (5) reported that most vegetable crops are sensitive to arsenic and that toxicity slowly decreased over many years. However, deeply rooted trees are apparently little affected by accumulations of arsenic at the cultivation depth. Ginsburg and Reed (6) found that high concentrations of DDT in the soil were detrimental to the growth of squash, snap beans, and some varieties of rye. Ackley *et al.* (1) reported concentrations of DDT as high as 81 p.p.m. in orchard soils and concluded that although the compound, beyond certain minimum concentrations, is toxic to plants, apple trees appear to be very tolerant to DDT in the soil.

The residual effects of the sulphur treatments were detrimental to beans in 1954, 1955, and 1956, and to carrots in 1955. The normal crop yields on the sulphur-treated plots in 1957 and 1958 reflect the lime treatments applied in the fall of 1956 and 1957. With the exception of the turnip crop, in 1954 yields on the parathion, ferbam-, BHC-, and chlordane-treated plots were not significantly different from those on the check.

Pesticide Content of Crops

Chemical analyses of plants grown in 1958 on soils treated with arsenic and on untreated soil are presented in Table 4. The results show the higher concentrations in the crops grown on the treated plots 5 years after treatments were discontinued, and also the distribution of arsenic in the plants. An examination of the carrot crop grown in 1955 to determine

TABLE 4.—ARSENIC CONTENT OF PLANTS—1958
(RESULTS IN P.P.M. AS—FRESH WEIGHT BASIS)

Crop	Plant part	Check plots	Arsenic plots
Peas	Seeds	0.01	0.18
	Pods	0.05	0.88
	Vines	0.12	2.14
Beans	Seeds	0.01	0.07
	Pods	0.27	0.79
	Leaves	0.21	1.92

the degree of translocation of the pesticides showed concentrations in the roots as follows: arsenic (As), 0.40 p.p.m.; DDT, 3.34 p.p.m.; BHC, 0.94 p.p.m.; parathion, 0.01 p.p.m. The DDT and parathion residues were located only on or in the surface scrapings of the roots; arsenic and BHC were found throughout the roots. BHC caused off-flavours in the carrots.

Effects of Pesticides on the Chemical Composition of the Soil

Results of chemical analyses of soil samples indicated that, with the exception of sulphur, applications of the various pesticides did not affect soil reaction, levels of exchangeable cations, or available phosphorus. In 1954, 1955, and 1956 soil acidity was significantly higher and exchangeable calcium and magnesium significantly lower in the sulphur-treated plots than in the checks.

There was a decrease in soil acidity and an increase in exchangeable calcium and magnesium in all plots as a result of the applications of dolomitic limestone in 1956 and 1957. However, in 1957 and 1958 differences between the sulphur-treated plots and the checks were still significant with respect to exchangeable calcium.

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LONG-TERM PERSISTENCE OF BHC, DDT AND CHLORDANE IN A SANDY LOAM SOIL

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ABSTRACT

Residues of technical BHC, DDT and chlordane present in a sandy loam soil in Nova Scotia 15 years after the last application were 7.5, 55 and 16%, respectively, of the amounts applied. BHC residues consisted of the alpha-, beta-, gamma- and delta-isomers at relative percentages of 36, 36, 16 and 12, respectively.

The beta isomer was the most persistent. DDT residues consisted chiefly of *p,p'*-DDT, *o,p'*-DDT and *p,p'*-DDE. Residues in chlordane-treated plots were principally alpha- and gamma-chlordane. There was little downward or lateral movement of these insecticides in the soil in 15 years.

INTRODUCTION

Field plots were established at Kentville in 1949 to study the long-term persistence of pesticides in soil and their effects on plant growth (Chisholm *et al.*, 1955; MacPhee *et al.*, 1960). Recently, with the acquisition of new equipment, it was possible to analyze these soils by gas chromatographic methods and determine both the isomers and breakdown products of the applied insecticides which were not detectable by colorimetric and titrimetric procedures. This paper reports on the residues of BHC, DDT and chlordane found 15 years after the last application.

MATERIALS AND METHODS

Pesticide plots were established on Berwick sandy loam (Chisholm *et al.*, 1955). Individual plots were 3.97×4.88 m and were separated by 1.53-m cultivated strips. The experimental design was a randomized block with four replications.

The insecticides, BHC, DDT and chlordane, were applied once annually in late May or early June before planting (Table 1). They were added to the surface of the plots in water suspension and then thoroughly incorporated into the soil to a depth of 15.2 cm with a rotovator. Each year from 1949 to the present the plots have been cultivated, and one or more of the following crops: beans, peas, oats, carrots, turnips, tomatoes, were grown. A 6-12-6 or 6-12-12 fertilizer was applied each year at 1120 kg/ha. Dolomitic limestone was applied at 5600 kg/ha in 1956 and at 2240 kg/ha in 1957.

On November 18, 1968, nine soil cores, 2.5 cm in diameter and 40.6 cm in length, were taken from each BHC, DDT and chlordane plot, divided into the 0-10, 10-20, 20-30 and 30-40 cm depths. The appropriate depths from each plot were pooled, air dried at room temperature and stored at 33 C.

Table 1. Insecticide treatments applied to plots

Treatment	Years applied	Formulation	Active ingredient	
			kg/ha/yr	ppm/15.2 cm ha/yr
BHC	1950-53	50% WP	29	13
DDT	1949-53	50% WP	117	52
Chlordane	1951-53	40% WP	14	5

Insecticide residues were extracted from soil samples with hexane/acetone (1:1 v/v) in Soxhlet extractors. The glass extraction thimble was Pyrex, 90 mm long \times 35 mm diameter, fitted with an "Extra Coarse" fritted glass disc. A 37-mm-diameter glass fiber filter paper was placed on the disc, then a layer of 3-mm glass beads, a 10.0-g soil sample previously moistened with 1 ml water, and a small pad of Pyrex glass wool. The sample was extracted with 300 ml solvent for 5 hours. Extracts were evaporated to dryness on a water bath at 40 C with a rotary evaporator, the residue redissolved in 10.0 ml hexane/acetone (1:1) and stored in the refrigerator.

A Micro Tek 200 gas chromatograph fitted with an electron-capture detector containing a 10 mc ^{63}Ni ionization source and operated at a potential of 20 volts was used. The column used was 1.83 m \times 0.64 cm Pyrex glass packed with 3% OV-17 on Gas-Chrom Q 100/120 mesh. Scavenger gas was purified nitrogen at 20 cc/min. Attenuation was usually 10×64 and detector temperature 275 C. Column temperatures for BHC, DDT and chlordane were 170, 190 and 185 C, respectively. Carrier gas flows were 65 cc/min, 65 cc/min, and 80 cc/min nitrogen, respectively.

Standard curves were prepared daily, for DDT and chlordane by plotting peak areas versus insecticide concentration and for BHC isomers by plotting peak heights versus concentration.

RESULTS AND DISCUSSION

BHC

Gas chromatography of soil extracts from BHC-treated plots showed the presence of four compounds corresponding to the alpha-, beta-, gamma- and delta-isomers of hexachlorocyclohexane (BHC). The identities of the four peaks were confirmed by gas chromatography on QF-1 and DC-200 columns and by thin-layer chromatography. Most of the BHC residues were in the 0–10 cm and 10–20 cm depths, with approximately equal concentrations at each depth. Below the 20-cm depth there was little BHC present (Table 2), indicating that leaching since the last pesticide application in 1953 had caused little downward movement of BHC. Analyses of soil samples taken outside the plot perimeters showed a small lateral movement of residues in the surface layers. This was attributed to cultivation.

The isomeric composition of the BHC soil residues in 1968 was considerably different (Table 3) from that reported for technical grade BHC (Ramsey and Patterson, 1946; Pennington and Meloan, 1967). The beta-isomer was the most persistent; 44% of that applied in 1950–1953 remained in the soil 15 years after

Table 2. Distribution of BHC isomers in 1968 in plots which received a total of 53 ppm technical BHC during 1950–1953 inclusive

Depth (cm)	Isomer, ppm*			
	Alpha	Gamma	Beta	Delta
0–10	0.89 \pm 0.17	0.41 \pm 0.09	1.12 \pm 0.05	0.38 \pm 0.05
10–20	1.09 \pm 0.30	0.52 \pm 0.13	0.85 \pm 0.13	0.39 \pm 0.09
20–30	0.17 \pm 0.13	0.08 \pm 0.06	0.11 \pm 0.03	0.04 \pm 0.02
30–40	tr†	tr	tr	tr

*Means of four replications with standard errors.

†Trace.

Table 3. Composition of BHC residues in 1968 compared with composition of material applied 1950-1953

BHC isomer	% Composition technical BHC	% Composition BHC soil residues, 1968	% of applied BHC isomer remaining in 1968
Alpha	70	36	4
Beta	6	36	44
Gamma	12	16	10
Delta	6	12	14

the last application. The alpha-isomer was the least persistent, although it was the most abundant isomer in technical BHC. Of the technical BHC applied to plots in 1950-1953, 7.5% (as the alpha-, beta-, gamma- and delta-isomers) remained in the soil in 1968. Analysis of BHC residues in these plots in 1958 (Chisholm, 1958, unpublished data) by a total chloride titrimetric method indicated that about 10% of the original applications remained then.

Nash and Woolson (1967) reported that in plots at Beltsville, Maryland, 10% of the applied BHC (as the alpha-, beta- and gamma-isomers) remained in a sandy loam soil 14 years after single applications at 25 and 100 ppm. These workers reported that BHC disappearance was a geometric function of time, BHC having a half-life of 2 years. The climate at Beltsville is warmer than at Kentville, which would tend to increase the loss of BHC by volatilization. However, their plots were not cultivated yearly, which would reduce the loss of BHC by volatilization. The value for residual BHC in the present investigation is thus in reasonably good agreement with theirs.

DDT

Analysis of extracts from the 1968 soil samples by gas-liquid chromatography showed the chief residues to be *p,p'*-DDT and *o,p'*-DDT, the principal constituents of technical DDT (Table 4). In addition, there were smaller amounts of *p,p'*-DDE and traces of *o,p'*-DDE and *p,p'*-DDD. These findings were confirmed by thin-layer chromatography (Thomas *et al.*, 1968). As with BHC, little DDT was found below the 20-cm depth and no lateral movement except a small amount due to cultivation was noted.

Since a sample of the applied DDT was not available for analysis, it was difficult to draw conclusions about the relative persistence of *o,p'*-DDT and *p,p'*-DDT. Technical DDT is said to contain up to 30% of the *o,p'*-isomer (Spencer,

Table 4. Distribution of DDT residues in 1968 in plots which had received a total of 262 ppm technical DDT during 1949-1953, inclusive

Depth (cm)	Compound, ppm*		
	<i>p,p'</i> -DDE	<i>o,p'</i> -DDT	<i>p,p'</i> -DDT
0-10	6.5±0.3	16.2±0.7	75±14
10-20	4.3±0.9	13.6±2.9	83±4
20-30	1.2±0.3	3.7±0.9	10±3
30-40	tr†	tr	tr

*Means of four replications with standard errors.

†Trace.

1968). It has been noted in a one-season experiment that *o,p'*-DDT disappears from soil faster than the *p,p'*-isomer (Sheets *et al.*, 1969). In the present investigation the relative percentage of the *o,p'*-isomer remaining in 1968 was 15, indicating that this isomer had disappeared faster, assuming that the initial proportions were 70% *p,p'*-DDT and 30% *o,p'*-DDT. The *p,p'*-DDE is a decomposition product of the *p,p'*-DDT originally added to the soil (Duffy and Wong, 1967). The trace amounts of *o,p'*-DDE present may indicate that little is formed from *o,p'*-DDT in soil, or that *o,p'*-DDE is less stable in soil than *p,p'*-DDE.

The small amount of *p,p'*-DDD present in the soil was probably a component of the applied technical DDT, although *p,p'*-DDD has been reported to be a metabolite of *p,p'*-DDT under anaerobic conditions (Ko and Lockwood, 1968).

Of the applied technical DDT, 55% (as *o,p'*-DDT, *p,p'*-DDT and *p,p'*-DDE) remained 15 years after the last application. Nash and Woolson (1967) reported an average of 39% DDT persisting after 17 years in three soils at Beltsville, Maryland.

Chlordane

Gas chromatography of extracts from chlordane-treated plots revealed fewer compounds than in technical chlordane, and presumably the more volatile and less stable compounds had disappeared. No sample of the technical grade chlordane applied was available for analysis, but two standard samples manufactured about 1951 and 1966 were compared by gas chromatography and found to be of almost identical composition. It can therefore be assumed that the material applied to the plots was very similar if not identical to present-day chlordane. Technical chlordane consists of about 9% heptachlor (Saha *et al.*, 1968) and 60 to 75% alpha- plus gamma-chlordane (Spencer, 1968). Saha and Lee (1969) have identified a number of other components.

After 15 years, alpha- and gamma-chlordane still formed the major portion of the soil residues. Heptachlor epoxide was noted as a minor component. Heptachlor had disappeared, as well as a few minor unidentified components.

Since the compounds missing from the weathered residues represented a minor proportion of the whole, it was decided as a working basis to express the soil residues as ppm chlordane by comparison with standard curves prepared from a standard sample of technical-grade chlordane.

As with BHC and DDT, most of the chlordane residues were found in the 0–10 cm and 10–20 cm depths (Table 5). There was little downward or lateral movement of this insecticide. Approximately 16% of the initial applications remained in the plots.

Table 5. Chlordane residues in 1968 in plots which received a total of 15 ppm technical chlordane during 1951–1953, inclusive

Depth (cm)	Chlordane, ppm*
0–10	1.9±0.2
10–20	1.4±0.1
20–30	0.3±0.1
30–40	tr†

*Means of four replications with standard errors.

†Trace.

Nash and Woolson (1967) reported that 40% of the applied chlordane remained 14 years after single applications of 100 and 50 ppm. Their higher rates of application would tend to give higher persistencies, as would their less intensive cropping.

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